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ERRATA AND AUTHORS' EMENDATIONS

Page 17, line 7, "at chlorosis" should be "a chlorosis "

Page 37, table 2, column 3, heading "4-lock bolts" should be "5-lock bolts "

Page 41, twenty-third line from bottom, omit the words "squares and" between "of" and "young "

Page 107, after "Materials and Methods" add footnote reference 3, at bottom of page insert the following as footnote 3 "Nitrogen equivalent indicated refers to grams per liter of NO_2 or NH_4 radical "

Page 132, table 3, footnote 1 should read "Hope/Ceres, N N 1127 "

Page 227, line 20 the words "and is soon completed" should appear between "(fig 7, B)" and "except" (line 19)

Page 233, line 11, "1928" should be "1828 "

Page 387, table 11, column 3, line 1, in place of levers insert "72.6", and in line following omit "31.5 "

Page 390, table 12, column 2, line 1, change "21.5" to "72.6 "

Page 405, in formula (1) $\frac{S(df)^2}{n^2}$ should be $\frac{(Sfd)^2}{n^2}$.

Page 647, legend for figure 2, "(See table 7)" should be "(See table 5)"

Page 732, in the first of the two equations in the final paragraph, the numerator of the right-hand member " $\sigma \cdot 12.3$ " should be " $\sigma \cdot 1.23$ "

Page 742, line 2, "In one pen" should be "In four pens "

Following page 760, plate 3, legend, line 4, "B" should be ".1 "

Page 770, Literature citation (15), "X 1" should be "X 3 "

Page 775, line 19, "5" should be "3 "

Page 788, line 3, "central Mexico" should be "Central America "

Page 861, 5th line from bottom, omit "and 1" after "B "

Page 913, table 1, column 1, line 5, "300 15 15 15 15" should be "300 15 15 15" "

Page 957, line 13, "rise 1 on" should be "fed "

Page 962, line 2, "milk-curd" should be "milk-curd "

Page 999, table 1, column 2, "Sporophores" should be "Sporophore "

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No. 1

FURTHER CYTOLOGICAL STUDIES OF HETEROTHALLISM IN PUCCINIA GRAMINIS¹

By RUTH F. ALLEN²

*Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry,
United States Department of Agriculture, and associate in agronomy, Agri-
cultural Experiment Station, University of California **

INTRODUCTION

An earlier paper (1)³ presented the results of a cytological study of the aecial generation of *Puccinia graminis* Pers. on the barberry (*Berberis vulgaris* L.). Stages of the entrance of the fungus and its development within the host plant were traced and an attempt was made to show its mode of sexual reproduction. *P. graminis* is heterothallic. Infections that are kept isolated remain gametophytic (with a few doubtful exceptions) and produce no aeciospores. The spermatia (pycniospores) function in effecting fertilization. The exact mode of entrance of the spermatia was not determined, but the frequent occurrence of cells with two or more nuclei in the walls of the spermogonium (pycnia) and in adjoining mycelium suggested the possibility that the paraphyses might serve to receive the spermatia. The sporophyte generation, once initiated, spreads from its point or points of origin through the mycelium. Aecia borne by such mycelium commonly contain sporophytic cells from their inception.

This paper presents the results of a more intensive study of the surface hyphae and related details in *Puccinia graminis*. For this study, slides were used that had been prepared during the earlier study and additional slides were made from material then embedded in paraffin.

INVESTIGATIONS

The rate of growth of the young infection varies greatly with environmental conditions and with the age of the host leaf. Four days after inoculation, under the most favorable conditions, the fungus has entered, has formed the primary hypha in a cell of the upper epidermis, has spread from this through the intercellular spaces of the mesophyll to the lower surface of the leaf, and has begun reproductive activities.

Plate 1, A, shows semidiagrammatically a cross section of a young barberry leaf, with thin-walled cells and small air spaces, that bears a typical well-grown 4-day infection. The leaf is drawn in outline only, and the fungus is shaded to bring it out more clearly. The hyphae have permeated the intercellular spaces of the mesophyll. At two

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² Acknowledgments are made to H. B. Humphrey, of the Division of Cereal Crops and Diseases, for careful reading of this manuscript and for barberry plants and spores; to W. P. Fraser, of the University of Saskatchewan, Canada, for spores; and to members of the Divisions of Agronomy and Genetics of the University of California for courtesies extended during the work.

³ Reference is made by number (italic) to Literature Cited, p. 14.

points at the upper surface (*a*, *b*) and one at the lower (*d*) young spermogonia are forming. At *c*, a hypha has grown up between cells of the upper epidermis and flattened itself against the cuticle. There are no hyphae in the stomata *d*, *e*, *f*, which appear to be closed, but at *e* a hypha is pushing down between a guard cell and the next epidermal cell.

A detailed study has been made of the hyphae that grow to the upper and lower surfaces of the leaf. Plate 1, B to G, shows typical surface hyphae found in 4-day infections. Plate 1, B, represents a detail at the upper surface of the leaf. Between the epidermis (*b*) and the palisade (*d*) there is a growth of mycelium (*c*, *e*) which is continuous at the right with a young spermogonium (beyond the limits of the drawing). From this subepidermal mycelium a hypha (*a*) has forced a passage up between the epidermal cells and has grown a short distance along the outer face of the epidermal cell beneath the cuticle. Plate 1, C, shows a similar surface hypha with a more extended subcuticular growth at *a*. Plate 1, D, *a*, shows a cross section of such a surface hypha at the lower epidermis. In all three (B, C, D) it is evident that the growing tip of the hypha has been able first to split the common wall between two epidermal cells at the middle lamella and grow out between the two to the surface and then separate the cuticle from the rest of the outer wall and force a passage there. Whether this is achieved by chemical or mechanical processes is not clear, but the latter seems probable.

At this time (four days after inoculation) hyphae are to be found about the stomata in the lower epidermis of the leaf, but rarely is one thrust between the guard cells. Plate 1, E, shows a bit of the lower epidermis, including a longitudinal section of a stoma (*b*). A hypha (*a*) is wedged firmly into the angle between the guard cell and the next epidermal cell. Another hypha (*c*), after growing down into the angle between the guard cells but failing to enter the stoma, has grown on to *d*. Perhaps the stoma is closed. Plate 1, F, shows a cross section of a stoma. One hypha (*a*) is pressed into the angle between the guard cells of the closed stoma, another (*b*) is forcing a passage between the guard cell and the next epidermal cell. The walls adjoining stomata seem to be a particularly vulnerable part of the young leaf's defenses. Plate 1, G, shows another stoma with a hypha growing down toward the lower surface at each end of the guard cell (*a*, *c*). At *b* is one of the very few examples of a hypha that has entered the stomatal opening.

These hyphae that emerge at the surface of the leaf resemble the receptive hyphae seen in other rusts (2, 3, 4, 5). At this stage, however, the infection, if visible macroscopically, is only a minute spot a

EXPLANATORY LEGEND FOR PLATE 1

A - Semidiagrammatic drawing of central section through 4-day infection of a barberry leaf by *Puccinia graminis*, fungus shaded. Young spermogonia at *a*, *b*, and *d*, surface hypha at *c*, hypha growing out between an epidermal cell and a guard cell of the adjoining stoma at *e*, hyphae near stoma at *f*. $\times 320$

B - Between the upper epidermis (*b*) and the palisade (*d*) are hyphae (*c*, *e*) from which a hypha (*a*) has grown up to the leaf surface and a short distance under the cuticle. Four-day infection. $\times 1,020$.

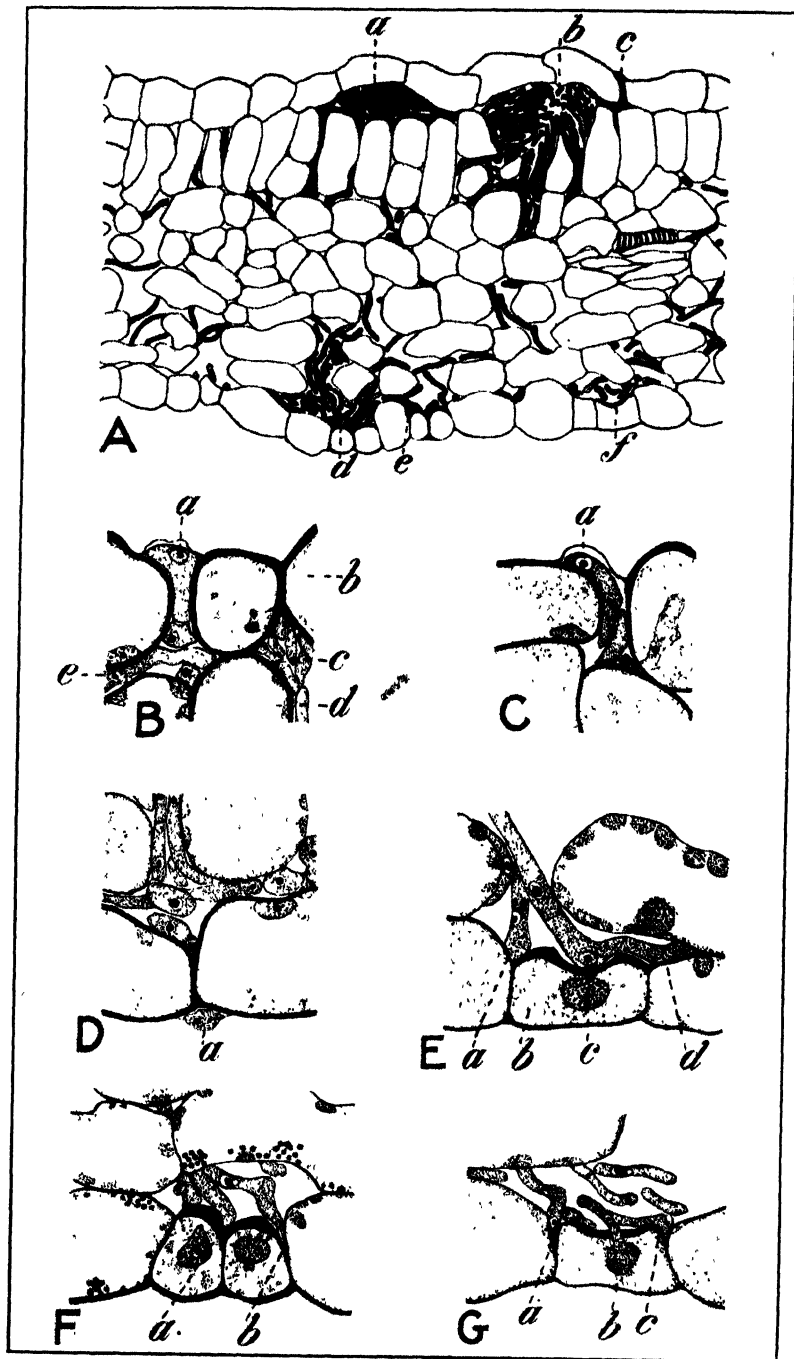
C - Similar to B, longer subcuticular growth at *a*. $\times 1,020$

D - Detail from 4-day infection. Cross section of subcuticular hypha at *a*. $\times 1,020$

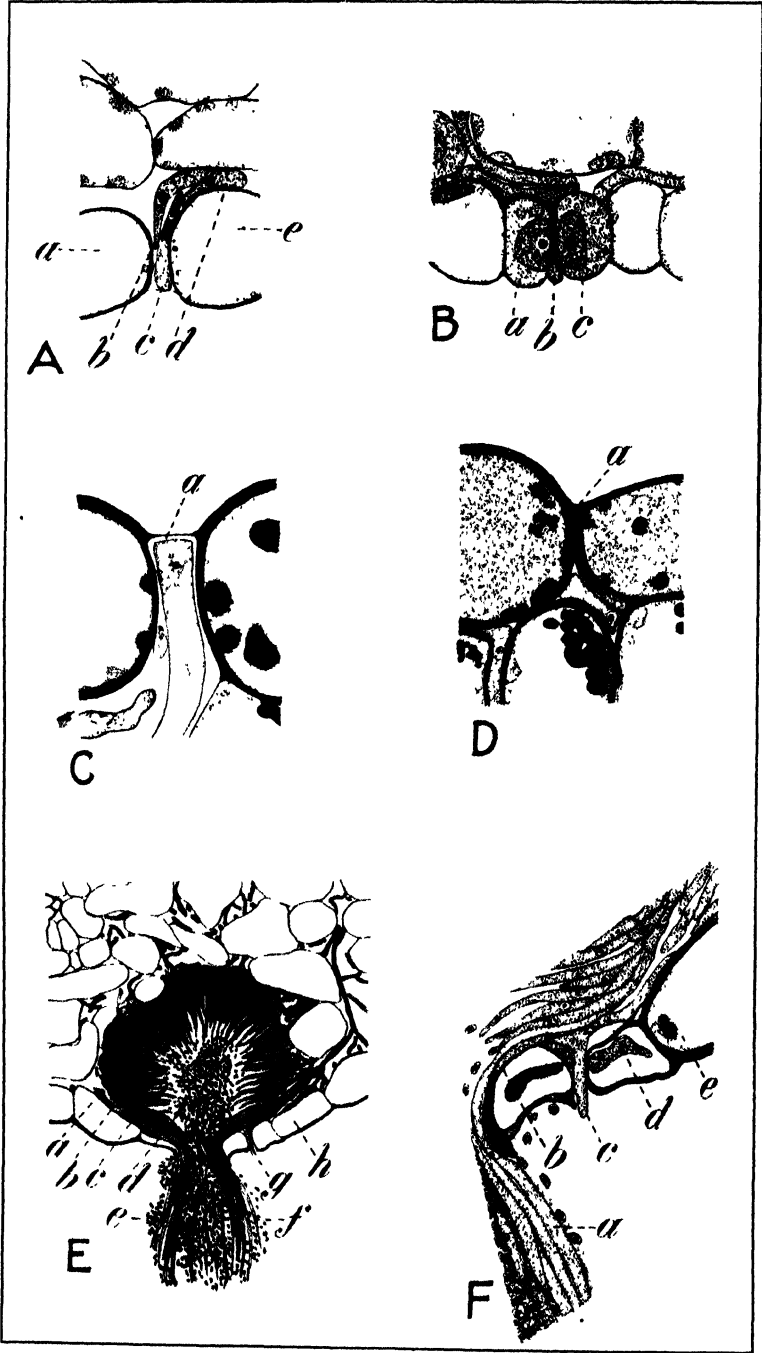
E - Detail from 4-day infection, showing longitudinal section of stoma (*b*). A hypha (*a*) is wedged into the angle at the end of the guard cell, another hypha has pressed into the hollow of the stoma at *c* and has then grown on to *d*. $\times 1,020$

F - Detail of lower epidermis of 4-day infection. A hypha (*a*) is wedged into the angle between the guard cells, another hypha (*b*) is forcing a passage outward at the side of the stoma. $\times 1,020$

G - Longitudinal section of stoma. Hyphae (*a*, *c*) are growing down at ends of guard cell; another hypha (*b*) is inserted in stomatal aperture. $\times 1,020$



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



shade lighter in color than the rest of the leaf. There are no mature spermogonia; consequently, there is no spermogonial exudate. There is nothing to attract the insects that might bring nectar from other infections and effect fertilization. These surface hyphae appear to be functional receptive hyphae, but there is small chance that fertilization would occur at this stage under natural conditions, and no case has been noted in the greenhouse material.

During the fifth and sixth days after inoculation the mycelium spreads farther in the mesophyll, the first spermogonia mature and open, and in a few of the most rapidly growing infections a rare aecial primordium forms.

If the infection is located in a young leaf with soft thin walls, the hyphae growing out to the surface remain unchanged. Plate 2, A, from a 5-day infection, shows a detail of the lower epidermis. Between the two epidermal cells *a* and *e*, a hypha (*c*) has grown down to the cuticle and other hyphae (*b*, *d*) are wedging in beside it. Plate 2, B, shows another bit of the lower epidermis. Between the still thin-walled guard cells (*a*, *c*) of the stoma, a hypha (*b*) has grown down flush with the outer surface of the leaf. This hypha is markedly constricted by the closing of the stoma.

When the young infection is growing in an older leaf, the thick, tough epidermal walls offer more resistance to the outward growth of hyphae. Under the conditions shown in plate 2, C and D, the formation of emergent hyphae obviously would be more difficult than in the examples already described.

Plate 2, C, *a*, shows a hypha that has grown up between two cells of the upper epidermis. The large size of the hypha and the conformation of the epidermal wall about its apex suggest that the hypha may have formed when the host walls were thin (compare with pl. 2, A) and that as the epidermal wall thickened it shaped itself about the hypha. In plate 2, D, on the contrary, a slender hypha (*a*) has worked its way out between the layers of the heavy epidermal walls almost to the surface and has then died.

Plate 2, E and F, shows another type of receptive hypha formed in connection with a spermogonium. Plate 2, E, represents semidiagrammatically a newly matured spermogonium. The interior portion consists of a nearly spherical shell or wall (*a*) from which numerous spermatophores (*b*) grow into the central cavity, and paraphyses (*c*) grow to the ostiole and out to form the extruded brush (*e*, *f*). Spermatia are set free into the central cavity (*d*) and ooze out through the ostiole to form the external exudate. The growing spermogonium lifts and stretches the epidermis adjoining it and puts it under strain.

EXPLANATORY LEGEND FOR PLATE 2

A—Detail of 5-day infection on a young barley leaf. Between two adjoining cells (*a*, *e*) of the lower epidermis a hypha (*c*) has grown down to the cuticle, other hyphae (*b*, *d*) are wedging in beside it. $\times 1,020$.

B—Detail of 6-day infection on young leaf. Between the guard cells of the stoma (*a*, *c*) a hypha (*b*) has grown down to the lower surface. $\times 1,020$.

C—Detail of 6-day infection on older leaf. A broad surface hypha (*a*) has grown between two cells of the upper epidermis. $\times 1,020$.

D—Detail of 6-day infection on older leaf. A slender hypha (*a*) has forced a passage up between heavy-walled epidermal cells and has then died. $\times 1,020$.

E—Newly opened spermogonium on lower surface of 6-day infection. Wall of spermogonium (*a*) gives rise to spermatophores (*b*) and paraphyses (*c*, *e*, *f*). Spermatia (*d*) are freed in central cavity and ooze out through ostiole. Surface hypha at *g*. Epidermal cell at *h*. $\times 320$.

F—Area from *f* to *h* of Plate 2, E, enlarged. Beneath the epidermis (*e*) are spermatophores and the bases of paraphyses which continue out to form the extruded brush at *a*. From the young paraphysis (*d*, *b*) a branch (*c*) has pushed out between epidermal cells to the leaf surface. $\times 1,020$.

Because of this weakened condition of the epidermis, hyphae frequently grow out between epidermal cells close to the ostiole (pl. 2, E, g).

The area *f* to *h*, in plate 2, E, is drawn enlarged as plate 2, F. At *a* is a portion of the extruded brush of paraphyses with the adherent exudate. At *d* a young paraphysis has forked, one branch (*b*) going on beneath the epidermis toward the ostiole, the other (*c*) growing out between epidermal cells to the surface. The hypha *c* may be regarded as either a receptive hypha or a displaced paraphysis.

The spermogonium just described opens upon the lower surface of the leaf. This is a common occurrence; a count of spermogonia in 7-day and 8-day material shows that 38 out of 117 (about one-third) open upon the lower side of the leaf.

In 7-day infections the surface hyphae are unchanged, except that there may be an increase in the subcuticular growth, particularly if the host leaf is young. Plate 3, A, shows a low-magnification diagram of a portion of a young barberry leaf. A detail at *c* is shown enlarged as plate 3, B. The hypha *a*, from the bases of the paraphyses, grew to the surface at *b* and along the surface for a considerable distance to *c*. It is not clear whether it is inclosed by the cuticle. Sometimes even more extended surface growth is found; and in at least one case it was two cells thick.

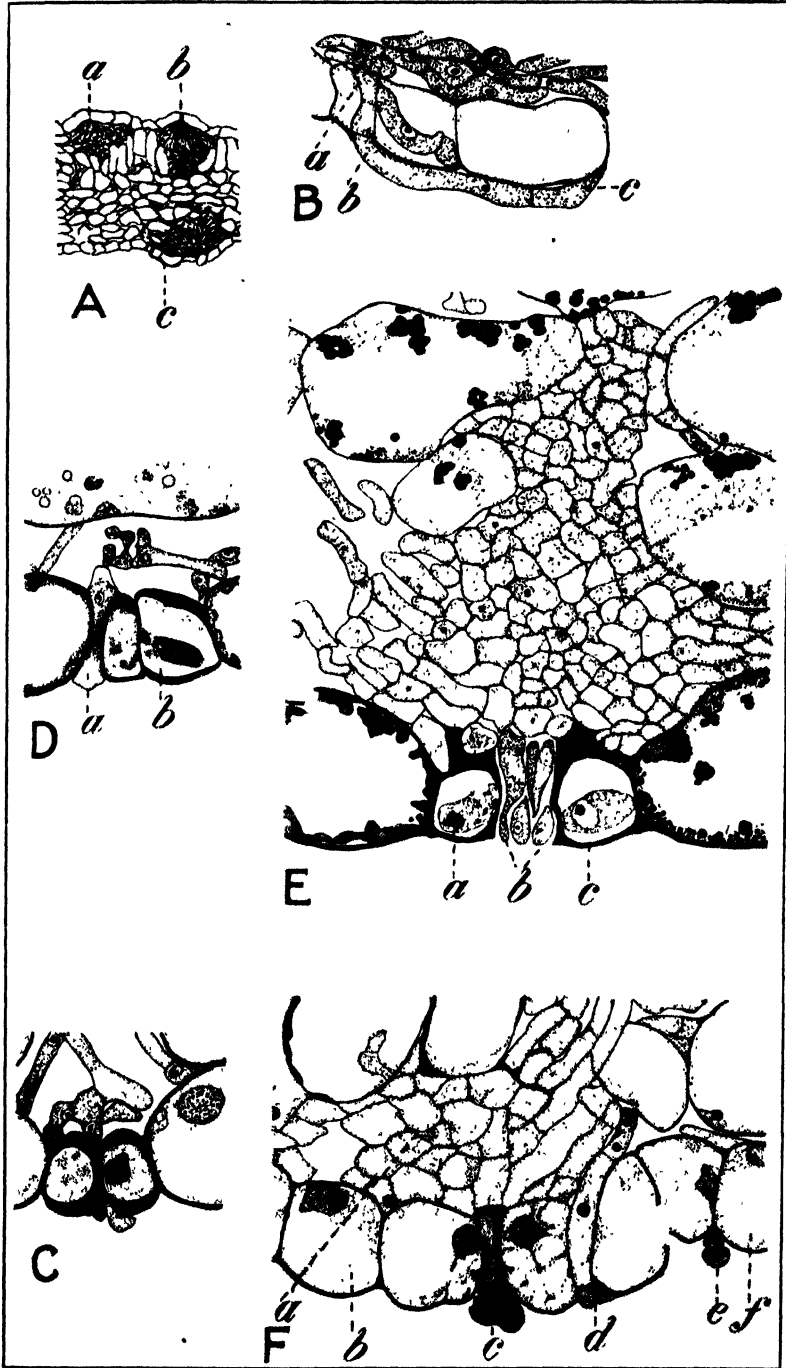
At this stage the stomata serve but rarely as outlets of surface hyphae. Plate 3, C, shows a stoma occupied by hyphae, but the shutting of the stoma has nearly pinched off the hyphae. More successful is the surface hypha at one end of the stoma. Plate 3, D, shows the hypha *a* between a guard cell (cut obliquely) and an adjacent epidermal cell.

To the statement that the stomata are of little importance, an exception should be made regarding stomata adjoining a spermogonium. Plate 3, E, drawn from an 8-day infection, shows a lateral section through a spermogonium, cut crosswise through the bases of paraphyses and spermatophores. The stoma (*a*, *c*) is wide open, perhaps because of the tension on the epidermis produced by the spermogonium, and in the stomatal aperture (*b*) are no less than five vigorous hyphae.

It has been noted that the progress of development of an infection varies considerably according to the age of the leaf in which the infection is growing, for older barberry leaves are very firm in texture and the epidermis becomes very heavy. Parallel studies have been made of two unfertilized 9-day infections, one growing on a fairly young leaf and the other on an old leaf. Table 1 shows the number and location of the receptive hyphae in the two infections.

EXPLANATORY LEGEND FOR PLATE 3

- A - Diagram of section of a barberry leaf through a 7-day infection. Young spermogonia at *a*, *b*, and *c*. $\times 115$
 B - Detail at A, *c*, enlarged, showing young paraphyses above the epidermis, from which a hypha (*a*) grows down to the outer surface at *b*, and along the surface to *c*. $\times 1,020$
 C - Bit of lower epidermis of 7-day infection, showing stomatal hyphae constricted by closure of stoma. $\times 1,020$
 D - Stoma (*b*), cut obliquely, from 7-day infection. A hypha (*a*) has grown down at one side of the stoma to the outer surface. $\times 1,020$
 E - Detail from 8-day infection. Stoma (*a*, *c*) forced wide open by pressure of growing spermogonium above it. Stomatal aperture (*b*) filled with hyphae. Inner walls of guard cells deformed. $\times 1,020$
 F - Detail from 9-day infection. Lateral section through spermogonium at *a*, above lower epidermis (*b*, *f*). Surface hyphae between epidermal cells at *c*, *d*, and *e*. $\times 1,020$.



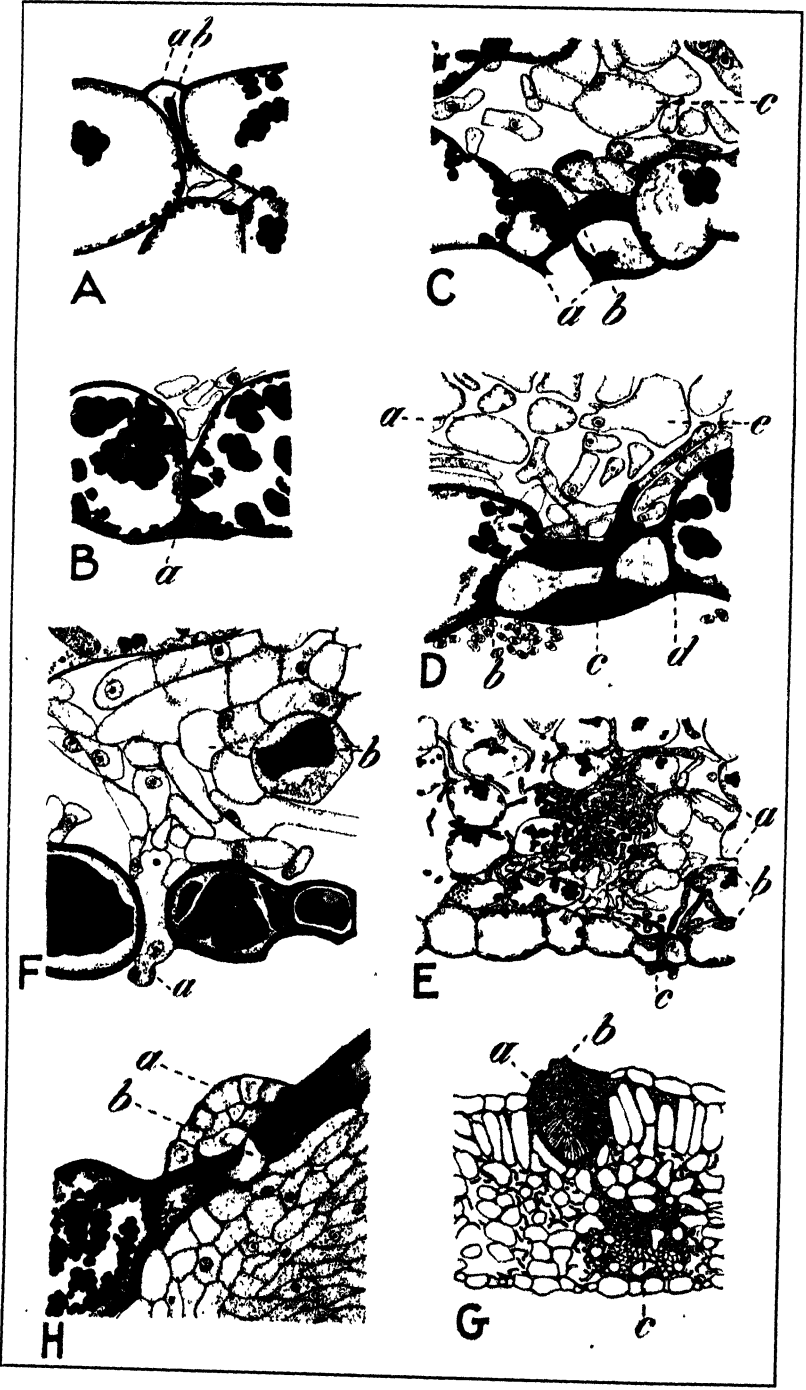


TABLE 1.—Number and location of receptive hyphae in two 9-day infections

Infection on—	Upper surface		Lower surface			Total
	Adjoining a spermatogonium	Remote from a spermatogonium	In stomata	Between epidermal cells	Remote from a spermatogonium	
Fairly young leaf	7	8	2	27	8	52
Old leaf	1	9	10	1	4	25

* Of these, 6 were located in stomata adjoining or near young aecia.

In the 9-day infection on the younger leaf, 37 out of 52 (about 70 percent) of the surface hyphae are at the lower surface of the leaf and 27 (about 50 percent) are between lower epidermal cells close to the ostiole of a spermatogonium. Plate 3, F, shows a typical example. The lateral section through the spermatogonium at *a* was cut across the bases of paraphyses and spermatophores. The lower epidermis (*b, f*) has been penetrated at three points. Hyphae branching from the paraphyses have pushed down between epidermal cells at *c, d*, and *e*, reaching the lower surface of the leaf. The one at *d* may be covered by the cuticle of the leaf, but, so far as can be discerned, the others are not. As in the other rusts studied (2, 3, 4), hyphae reaching the leaf surface are short-lived. Only the one at *d* is still living.

In the 9-day infection on the older leaf (Table 1) the surface hyphae differ in number, distribution, and condition from those on the younger leaf. Only 25 surface hyphae were found—less than half as many as on the younger leaf. Moreover, all 25 were dead—not one, so far as can be judged by the appearance, was in condition to function.

Typical examples from this 9-day infection have been drawn. Plate 4, A, shows a hypha (*b*) that pushed up between cells of the upper epidermis, then died and shrank back part way. A definite layer of cuticle covers it at *a*. Plate 4, B, shows a bit of the lower epidermis with a short subcuticular hypha (*a*), now dead and inclosed on all sides by a thick layer of cuticle.

Ten of the surface hyphae of this infection (table 1) occurred at the lower surface in stomata. Even more of them attempt to enter stomata and fail. Plate 4, C, shows a stoma (*a*) and above it, at *c*, the large loose cells of the "space-making" part of a young aecium.

EXPLANATORY LEGEND FOR PLATE 4

A—Detail from upper epidermis of 9-day infection. A hypha (*b*) has grown up to the outer wall (*a*) from subepidermal mycelium and then died. $\times 1,020$

B—Detail of lower epidermis from 9-day infection. A portion of a subcuticular hypha is embedded in heavy walls at *a*. $\times 1,020$.

C—Heavy-walled closed stoma at *a*. Above it at *b* is a hypha unable to enter the stoma, aecial cells at *c*. Nine-day infection. $\times 1,020$.

D—Longitudinal view of guard cell (*b, d*) in epidermis beneath aecium (*a, c*). Dead stomatal hypha (*c*). Nine-day infection. $\times 1,020$.

E—Aecial primordium from 8-day infection showing a dense upper area (*a*), a less dense lower area (*b*) and dead hyphae in the stoma at *c*. $\times 320$.

F—Detail of aecial primordium (*b*) of a 10-day infection. A living surface hypha in lower epidermis at *a*. $\times 1,020$.

G—Diagram of section through 10-day fertile infection, showing spermatogonium (*b*), external fungous growth (*a*), and young fertile aecium (*c*). $\times 115$.

H—Detail from G at *a*, enlarged, showing external fungous growth (*a*) connecting with spermatogonium at *b*. $\times 1,020$.

The guard cells of the stoma possess thick walls and the stoma is tightly closed. A hypha is pressed into the angle between the guard cells at *b*, but can not effect an entrance. This is of common occurrence. Under these conditions relatively few of the stomata are occupied by hyphae, and when a hypha does enter it soon dies. Plate 4, D, shows a longitudinal section of a guard cell (*b, d*) with aecial cells above it (*a, e*). A hypha (*c*) grew into the stoma but is now dead.

The earliest aecial primordium noted was in a 6-day infection. Aecial primordia are of rare occurrence, however, during the first week of development of the rust; on the eighth day after inoculation they become more common. Plate 4, E, shows one at a low magnification from an 8-day infection. It consists of a tangle of hyphae in the mesophyll near the lower surface of the leaf. The upper part of the aecial primordium (*a*) consists of small dense cells, and the lower part (*b*) is made up of larger vacuolated cells more loosely spaced. It is gametophytic throughout; the cells are uninucleate. At *c* is a stoma occupied by hyphae, now dead. These are the only surface hyphae adjoining this aecial primordium, and they appear incapable of functioning.

A number of similar aecial primordia have been examined. Some show no surface hyphae in the contiguous epidermis; others have one or even two, either between epidermal cells or in stomata. These surface hyphae often are dead. Occasionally, however, one is living and apparently capable of functioning, as in the example drawn from a 10-day infection in plate 4, F, at *a*.

Aecial primordia (pl. 4, E, *a, b*) consist of a loose snarl of hyphae centered a short distance above the lower epidermis. They exert no appreciable pressure on the lower epidermis. Even when somewhat larger and more massive and when the mesophyll cells included within them are partly crushed, the contiguous lower epidermis is not noticeably bulged. By the twelfth day after inoculation, however, the growing potential aecia, whether fertile or sterile, exert marked pressure on the lower epidermis, stretching it and tending to force open the stomata. Moreover, in well-grown 12-day infections the infected leaf tissues are becoming hypertrophied. Palisade and mesophyll cells are expanding rapidly and the infected part of the leaf may have doubled in thickness. This puts additional stress upon the epidermis, which incloses the expanding tissues. At the same time the epidermis, especially the upper epidermis, is becoming tougher and more impenetrable as the leaf matures.

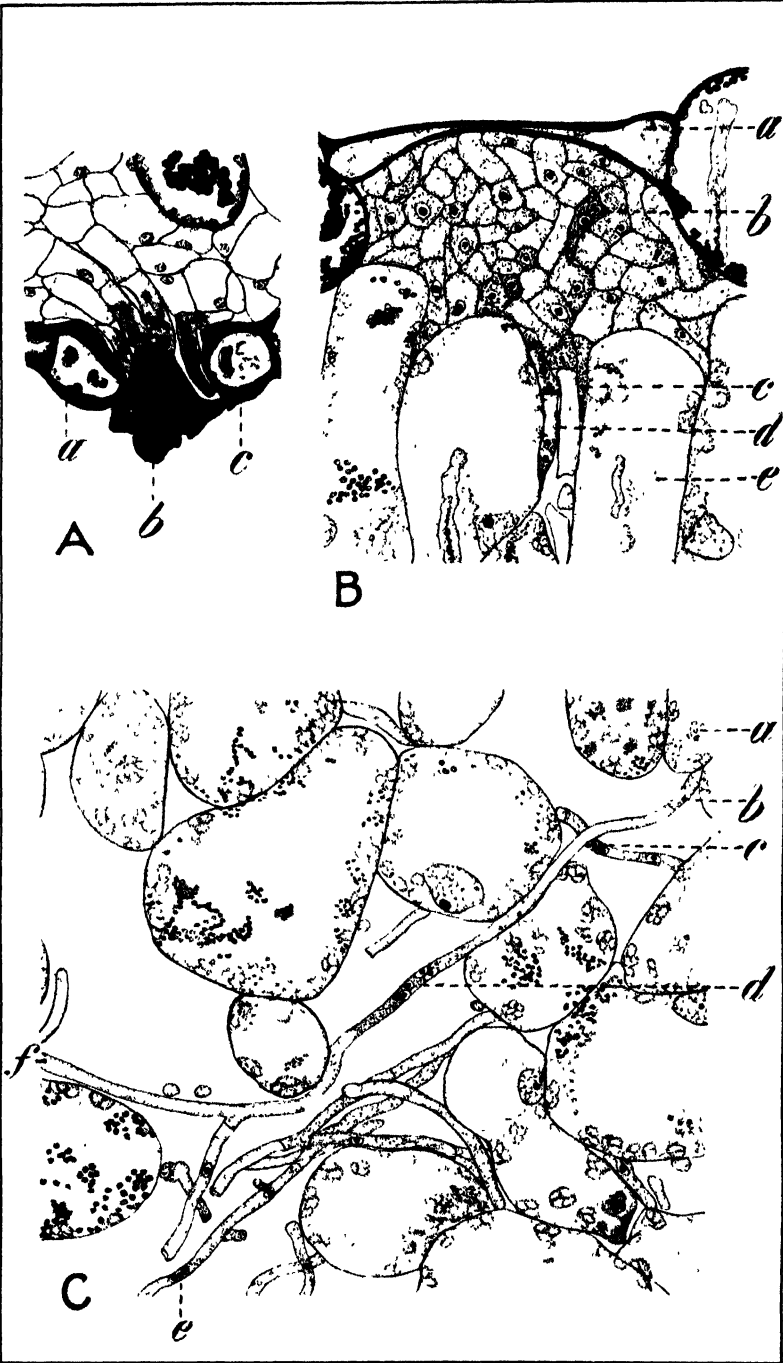
These conditions produce a marked change in the formation of surface hyphae. As the epidermal walls thicken, the hyphae that have pushed out between epidermal cells die and disappear. Steps in this process are seen in plate 4, A and B, and soon after this these hyphae become indistinguishable. The formation of new hyphae between epidermal cells becomes increasingly difficult as the epidermal

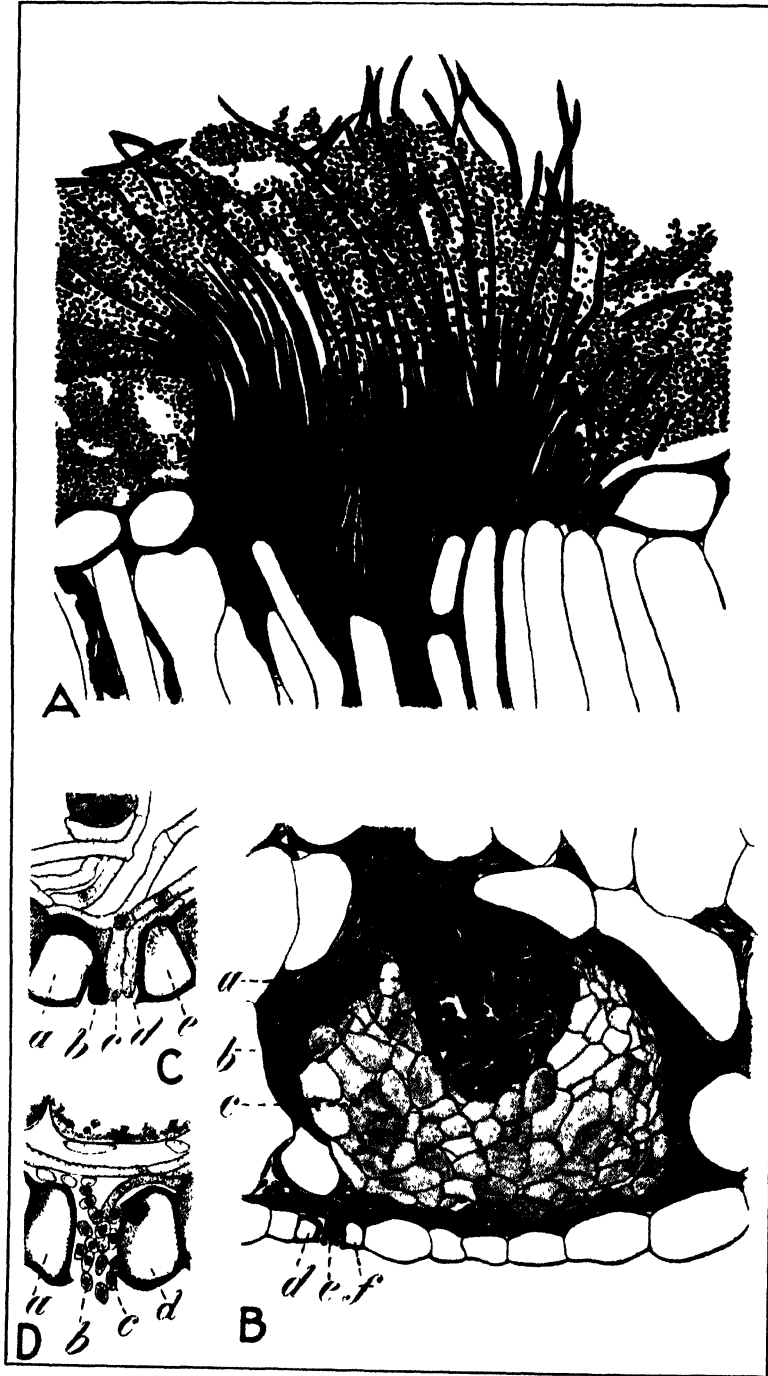
EXPLANATORY LEGEND FOR PLATE 5

A —Detail of 51-day infection, showing the widely separated guard cells of a stoma (*a, c*) filled with a group of receptive hyphae (*b*), now dead. $\times 1,020$

B —Detail from 10-day infection. Between the flattened upper epidermis (*a*) and the palisade (*e*) is a lateral section through a spermatogonium with a trinucleate cell (*b*) among the roots of the paraphyses, and sporophytic hyphae (*c, d*) growing downward from them. $\times 1,020$.

C —Portion of infection below the spermatogonium in B, showing lower end of palisade (*a*) a hypha (*b f*) containing 5 nuclei at *d*, and other sporophytic hyphae at *c* and *e*. $\times 1,020$.





walls become heavier. One result of this is a rapid decrease in the number of hyphae at the upper surface. At the same time the stomata (which, in the barberry, are on the dorsal surface of the leaf) are under increasing tension and are more easily opened; they soon become crowded with hyphae.

Table 2 shows the results of studies of the number and location of surface hyphae in older sterile infections. There were very few receptive hyphae left on the upper surface and these were in the disturbed areas at the ostioles of spermogonia. Over 95 percent of the surface hyphae were in the lower epidermis, some adjoining aecia, others near spermogonia, and still others in between. Nearly all were in stomata. In the oldest infection studied (51-day), 214 out of the total 220 surface hyphae were in stomata. In many cases a stoma was stretched wide open (pl. 5, A, *a*, *c*) and crowded with half a dozen hyphae (*b*). Nearly all these hyphae were dead, for stomatal hyphae are short-lived.

As noted earlier (1, p. 598), in isolated infections in which fertilization is prevented the spermogonia (pycnia) remain active for a surprisingly long period. The drop of spermogonial exudate on the upper surface of the leaf is maintained as long as the fungus lives. These long-lived spermogonia undergo marked secondary changes. The original paraphyses wither and new ones form from deeper parts of the spermogonial wall. Even the spermatophores may elongate, taking on the appearance of paraphyses, and the whole spermogonium opens out. The extreme case shown in plate 6, A, as compared with the newly matured spermogonium shown in plate 2, E (both of which are drawn at the same magnification), indicates the extent of these secondary changes. In instances where fertilization obtains, whether it takes place early or late, the formation of spermatia ceases, the exudate dries, and the spermogonia die.

TABLE 2.—Number and location of surface hyphae in older sterile infections of different ages

Age of infection	Upper epidermis (at spermogonia)	Lower epidermis				Total
		Adjoining aecia	Adjoining spermogonia	Between aecia and spermogonia	In stomata	
14 days	1	25	3	22	41	51
20 days	2	9	31	66	106	108
51 days	1	133	5	81	214	220

There is a relatively small amount of spermogonial exudate on the lower surface of infections—nearly all is on the upper surface, where it would be most easily seen by flying insect visitors. Yet if insects should transfer nectar to the upper surface of older sterile infections, fertilization could not be effected through ordinary surface hyphae,

EXPLANATORY LEGEND FOR PLATE 6

- A.—Wide-open, active spermogonium showing marked secondary changes. Fifty-one day infection $\times 320$.
 B.—Fertile aecium from 12-day infection, showing spore chains (*a*), spermogonial wall (*b*), and space-making area (*c*). Below is a wide-open stoma (*d*, *f*) filled with hyphae (*e*). $\times 320$.
 C.—Stoma (*a*, *c*) filled with hyphae (*b*, *c*, *d*). Twelve-day infection. $\times 1,020$.
 D.—Stoma (*a*, *d*) stomatal hypha (*c*), and spermatia (*b*). Twelve-day infection. $\times 1,020$.

for the upper leaf epidermis has long been impermeable to hyphae. The only way in which fertilization could occur at the upper surface would be through the spermatogonia themselves.

On the lower surface, on the contrary, there is a steadily increasing number of stomatal hyphae in aging sterile infections. These presumably could serve to receive spermatial nuclei. It should be added, however, that these stomatal hyphae do not become common until the aecial primordia are past the most favorable stage for the initiation of the sporophyte.

It may be that fertilization can take place at any time when fresh spermatia of one sex are placed in contact with living hyphae of the other sex. In the material at hand, however, it has been rare to find sporophytic mycelium (evidence that fertilization has taken place) before the ninth day after inoculation.

In an earlier paper (1) an instance was given suggesting one means of fertilization. In an open spermatogonium at the upper surface of the leaf, fungous cells with two and three nuclei were found at the base of the paraphyses (1, *pl. 11, C and D*) and in hyphae leading downward from them. The successive sections of this spermatogonium have been reexamined. There is no break in the epidermis around the spermatogonium. There are no surface hyphae between epidermal cells anywhere near the spermatogonium. The only means of entrance for spermatial nuclei is through the spermatogonium itself.

In active spermatogonia new paraphyses continue to form; these fresh young paraphyses may serve as receptive hyphae, the spermatial nuclei entering them and migrating down through them to the cells of the spermatogonial wall at their base. Further evidence along this line has been found. Plate 5, B, shows a lateral section through a spermatogonium that includes a cross section through the bases of paraphyses and spermatophores. Above it is a much-flattened epidermal cell (*a*) and below it the palisade layer (*e*). At *b*, in the central area of the fungous mass, is a trinucleate cell that has begun to grow downward, and at *c* and *d* are sporophytic hyphae growing down between palisade cells.

The mesophyll tissue just below this spermatogonium is shown in plate 5, C. Leading down from the inner end of the palisade cell at *a* is the hypha *b f*, containing a group of five nuclei in its central region at *d*. Appearances here suggest that these nuclei and the cytoplasm about them are migrating down the hypha. Certainly here is no ordinary growth of a sporophytic hypha with regular binucleate cells and a conjugate division at the formation of each new cell. Other sporophytic cells are found at *c* and *e*.

Cases of this sort have been observed repeatedly, where the sporophyte appears to have arisen in a spermatogonium and spread from there either by new sporophytic growth or by the migration of nuclei through already existing hyphae.

No unmistakable case has been found in which the sporophyte started in a separate surface hypha between upper epidermal cells. Usually the great majority of these hyphae have died and disappeared by the time fertilization ordinarily takes place. Unless a barberry leaf is exceptionally young when inoculated with the rust, it has made good progress toward maturity by the tenth day following, and its epidermis is nearly or quite impenetrable to any hypha.

Mention has been made of a surface growth of hyphae two cells thick. An example of a surface growth three cells thick is shown in plate 4, G and H. Plate 4, G, is a diagram of a 10-day infection magnified 115 times. At *b* is a slightly lateral section through a mature spermogonium, and at *c* a section through a fertile aecium. At *a* is a close group of hyphae outside of the epidermis. This detail is drawn enlarged as plate 4, H. The fungous growth (*a*) is three cells thick. At *b* it connects through the epidermis with the spermogonial hyphae. This external strand of hyphae runs through a whole row of sections, then forks into two lesser strands which disappear. It is not known whether such a growth as this can serve as an avenue of entrance for spermatial nuclei.

Fertilization had occurred in this infection a day or two before fixation. There were a few binucleate cells in the wall of the spermogonium, in the surrounding mycelium, and scattered all through the aecium (*c*); it was too late to determine, however, whether the spermatial nuclei entered at this external fungous growth or at the paraphyses of the spermogonium.

A count was made of the surface hyphae in three fertilized 10-day infections on the leaf from which the drawings shown in plate 4, G and H, were made. The total number of hyphae at both leaf surfaces for the 3 infections was 65, of which only 10 were unmistakably living. Ten of the sixty-five were at the upper epidermis; 8 between epidermal cells near the ostioles of spermogonia (the most vulnerable place), and only 2 in locations remote from spermogonia. Fifty-five were at the lower surface; 6 at spermogonia, 17 near aecia, and 32 not located at either a spermogonium or an aecium. Comparatively few of these 55 were between ordinary epidermal cells. Nearly all were in stomata, this apparently being the only feasible means of egress, and nearly all were dead. They may have been killed by the closing of the powerful thick-walled guard cells of these little stomata. In fact, a few dead pinched-off bits of hyphae may be seen outside the stoma.

A study was made of another 10-day fertilized infection, which for some unknown reason had not made a good growth but in which a few open spermogonia and young aecia were observable. A careful survey of both upper and lower epidermis revealed not a single receptive hypha, living or dead. This exceptional situation affords valuable confirmation of the hypothesis that spermogonia may serve as avenues of entrance for spermatial nuclei. In this case the spermogonia were the only means of entrance, yet there were occasional binucleate cells in the walls of the spermogonia, in the mycelium, and in at least one of the aecia.

In the fertile infection, as in the sterile, when the aecia grew larger and host tissues became hypertrophied there was a rapid increase in the number of wide-open stomata filled with hyphae. Plate 6, B, shows semidiagrammatically a detail from a 12-day infection. This leaf was exceptionally young when inoculated, as evidenced by the fairly thin walls of the epidermal cells. The fungus grew rapidly in the young tender leaf, forming numerous spermogonia, receptive hyphae, and aecia. Fertilization occurred on the ninth day, three days before the material was fixed. One of the fertile aecia is shown in plate 6, B. The young spore chains at *a* (not a median section) are well established. Below, the stoma (*d, f*) is wide open and filled with hyphae

(e). There are many such stomata. Surface hyphae have formed at 96 points in this infection, of which 57 are at stomata.

Plate 6, C, shows another of the occupied stomata of this infection. Between the guard cells (*a*, *e*,) are three hyphae. The oldest (*b*) evidently developed while the stoma could still open and shut, for this hypha is pinched in the middle and the part exterior to the constriction is dead. The two younger hyphae in the same stoma (*c*, *d*) show no such constriction and evidently formed after the stoma had become inactive.

A third stoma of this infection (pl. 6, D) is of greater interest, for here, in addition to the single receptive hypha (*c*) in the wide-open stoma (*a*, *d*), there are a dozen or so spermatia at *b*. There is no proof that fertilization occurred at this point, for the adjoining mycelium is gametophytic. In fact, these spermatia may have been produced by this same infection and so may be incapable of fertilizing it. Fertilization, however, could probably take place in this way if spermatia from another infection of opposite sex were placed there. To be sure, stomatal hyphae do not form in any abundance in *Puccinia graminis* until after the first aecia have passed the stage most favorable for the initiation of the sporophyte, but new aecia continue to form for a few days afterwards.

All attempts to find proof of fertilization through stomatal hyphae have failed. This was to be expected. The barberry plants used in this work were grown in the greenhouse. They were protected by cages made of light wooden frames covered by tarlatan to prevent visits from flying insects. Moreover, they were remarkably free from aphids and thrips, which visit the lower surfaces of leaves in other plants. It seemed possible that barberry leaves are too tough for such insects or that some chemical quality of the leaves repels them. Under such circumstances, no fertilization could be brought about by insect carriers of spermatogonial exudate. At the time of these experiments (1928) it was not known that fertilization could occur at the lower surface of infected areas. When nectar was transferred during the experiments, it was placed upon the upper leaf surface at infected spots. Fertilization would have occurred at the lower surface only by accident. In view of what is known of other rusts, however (3, 4, 5), it seems probable that fertilization through stomatal hyphae can occur when conditions are favorable.

DISCUSSION

Heterothallism in *Puccinia graminis* and the function of the spermatia (pycniospores) in effecting fertilization have been adequately demonstrated by the experiments of Craigie (8, 9, 10, 11). The ability of the physiologic forms of *P. graminis* to form hybrids on the barberry, resulting in the production of new physiologic forms, has been demonstrated by Waterhouse (26), Newton, Johnson, and Brown (22, 23), and Stakman, Levine, and Cotter (24).

The means by which spermatia enter remained unknown until Andrus (5) discovered in *Uromyces appendiculatus* (Pers.) Fries hyphae which grow to the surface of the leaf and into which spermatial nuclei might enter directly. The initiation of the sporophyte by the entrance of spermatial nuclei into hyphae at the surface of the host tissue is now known for the following rusts: *U. appendiculatus*, *U.*

vignae Barclay (5), *Puccinia triticina* Eriks. (2, 3), *P. coronata* Cda. (4), and *P. graminis*. This method probably will be found to be much more widespread; how much more is, at present, only a matter of conjecture. Andrus cites several cases in which authors have quite incidentally figured or described surface hyphae. The resemblance between Kursanov's drawings of *Aecidium punctatum* Pers. (17, p. 13), and plate 2, A, and plate 4, F, of the present paper, is too close to need further comment.

Only half a dozen rusts are known to be heterothallic, but there is little question that heterothallism will be found in many others. There is, however, much adverse evidence. A long series of papers on the cytology of the aecial generation of rusts, published before the discovery of heterothallism and adequately summed up in several papers (1, 6, 12, 16, 17), presents evidence that the sporophyte generation is initiated, not at the surface of the host plant but in the sporogenous area of the aecium, by fusions of cells of adjoining hyphae of the same individual or even of two cells of the same hypha. This viewpoint seems incompatible with the results of recent investigations in this field. Only by a detailed study of representative rusts, both long-cycle and short-cycle, from the different rust groups can it be determined whether heterothallism and a method of fertilization through spermatia and receptive hyphae are of general occurrence.

In this connection the remarkably constant presence of spermogonia (pycnia) just before the advent of the sporophyte generation in rusts has been recognized as significant. Jackson (16, p. 98-99) notes--

that the pycnia are quite constantly associated with long-cycled species and that they are omitted most commonly in those which give some evidence of being in an unstable condition as to life history and in short-cycled species or those of reduced life cycle. If the pycniospores exercise the function indicated by Craigie's work, then it would appear quite probable that they would be functionless in homothallic species and might soon be dropped from the life cycle.

He ventures the generalization that--

the fact that pycnia are so universally present in normal heter- and autoecioforms suggests that heterothallism may be a primitive character in the rusts and the usual condition in the present long-cycled species.

The age of the barberry leaf, which serves as host, affects the development of *Puccinia graminis* at more than one point. It had already been known that the entrance of the germinating sporidium may be prevented by the heavy outer epidermal wall of older leaves. Melhus, Durrell, and Kirby state (21, p. 297-298):

When the leaves of *Berberis vulgaris* have taken on a dark green color and crisp, hard appearance, infection apparently does not take place. * * * The marked difference in the susceptibility of young and old leaves of the barberry may be due to the thickness of cuticle and epidermis, considering that infection is accomplished by penetration of the tissues and not through invasion of stomata.

Following up this question, Melander and Craigie (20), in studies of the comparative resistance of different species of barberry to stem rust, measured the thickness and resistance to puncture of the outer epidermal walls of leaves of different ages. They found that the species of *Berberis* whose leaves possess epidermis that is very resistant to puncture usually are resistant to rust, and state (p. 99): "But the leaves of susceptible species also become practically immune with age. * * * Leaves of *B. vulgaris* 10 days old are fairly resistant to *P. graminis*."

Other instances are known in which the entrance and progress of a parasite are held in check by the firm texture of the host tissues. Vallean (25) and later Willaman, Pervier, and Triebold (27) studied the varietal resistance of plums to brown rot and found that resistance was at least partly mechanical. Resistant varieties differ from susceptible varieties in possessing a tougher cuticle, corky walls about the stomatal cavities, higher fiber content, and firmer flesh. Hursh (15) describes the sharp limitation placed upon the spread of mycelium of *Puccinia graminis* in the stems of wheat varieties with abundant sclerenchyma. Goulden (13) discusses the possibilities of "mature plant resistance" (due to the development of abundant sclerenchyma) in breeding for resistance to *P. graminis*. And Goulden, Newton, and Brown (14) report varietal tests of mature-plant resistance looking toward that end.

Less has been said about the possibility that the tough epidermis of a leaf may interfere with the reproductive processes of a parasite already established within it. There must, however, be many cases like that reported by Kusano (18). He studied the resistance of *Oenothera* species to the attack of *Synchytrium fulgens* Schroet. and found that the thick, tough epidermis of *Oenothera odorata* Jacq. often prevented infection, and that when infection did take place the fungus developed normally but that "the mature sorus was sometimes incapable of dehiscing and ultimately turned brown inside the host-cell" (18, p. 314).

In *Puccinia graminis*, if the barberry leaf is young when infected, reproduction is unrestricted and tends to be of the type found in the other cereal rusts. If the leaf is somewhat older, the infection grows and spermogonia form and break through the epidermis; but receptive hyphae form with difficulty, and fertilization depends upon the spermogonia or later upon delayed receptive hyphae in old wide-open stomata. If the leaf is still older at the time of inoculation, infection takes place; but the mycelium remains minute, causes little or no hypertrophy of the host, and does not form reproductive structures. If the leaf is very old no infection takes place.

The terminology of the reproductive processes in rusts remains a vexed question. Genetically, as Craigie (9, 10, 11) clearly proved, a (+) sporidium develops into a (+) gametophytic mycelium, which bears (+) spermogonia and spermatia, while a (-) sporidium grows into a (-) gametophytic mycelium with (-) spermogonia and spermatia. On that basis, the spermatia of a male individual are male and can function only when transferred to a female infection, whereas the spermatia of a female infection are genetically female and can function only when transferred to a genetically male individual. If there were no other data available, there would be small argument for the use of the words "spermogonium" and "spermatia," as these are names properly applied only to male structures. It is with some hesitation, therefore, that one continues to use the word "spermogonium" for a structure that can and does perform both male and female functions.

Consideration must be given, however, to the discovery by Andrus (5) of hyphae that grow to the surface of the leaf and serve to receive spermatial nuclei. If, as Andrus believes, these hyphae grow from "eggs" in the fertile area of young aecia and are to be considered

"trichogynes," there may be at least a morphological reason for the use of male and female names for the reproductive structures, although no genetical basis has been found.

So far, studies of heterothallism in cereal rusts afford little support for a belief in trichogynes. Hyphae grow to the surface of the leaf several days before the first aecial primordium appears. Some of the surface hyphae that form later have no direct connection with the aecia, except in so far as any monosporidial mycelium constitutes a connected system of hyphae. Even in a species in which a direct connection between receptive hyphae and aecia is of common occurrence, as in *Puccinia triticea* (2, 3), the receptive hyphae form in stomata from ordinary mycelium and aecial primordia then develop around them. The sporogenous area of the aecium takes shape a day or two later. In the cereal rusts, therefore, small justification has been found for the use of the words "egg" and "trichogyne."

In general, a single gametophytic mycelium bears both spermogonia and receptive hyphae. In other words, an individual whose cultural data indicate it to be genetically unisexual bears both the cells to be transferred to other infections and the hyphae that will receive cells from other infections. Genetically, it is unisexual; functionally, it is bisexual. In *Puccinia triticea*, however, there is some degree of segregation. Some individuals bear chiefly spermogonia, and others bear chiefly receptive hyphae and aecia. In a former paper the writer (2) has stated: "The infections of *Puccinia triticea* can be arranged into a series with an almost exclusively spermogonial type at one end and a completely aecial type at the other." Although it has not been proved to have a genetic foundation, this unequal distribution of spermogonia and receptive hyphae seems to offer added justification for the use of a sex terminology for these organs.

At the other extreme is the condition found in *Puccinia graminis*. When the texture of the leaf permits, receptive hyphae form between epidermal cells in *P. graminis*, as in the other cereal rusts. When the epidermal walls of the leaf become so tough that the hyphae can not force their way out, fertilization, at the upper epidermis at least, can take place only through the spermogonium itself.

From the point or points of its origin on the leaf surface, the sporophyte spreads rapidly through the mycelium and invades the aecial primordia. There is some evidence here, as in the other rusts studied (2, 3, 4), that the spread of the sporophyte is achieved, at least partly, by nuclear migrations. The group of five nuclei in the hypha in plate 5, C, d, is typical. In the earlier work on *Puccinia graminis*, it was found that irregularities in the number, size, and location of nuclei within a cell were common, and that successive cells frequently contained different numbers of nuclei (1, pls. 13, C, and 14, A). There is no means of watching the actual migration of nuclei, but the observed facts may be explained on the assumption that introduced nuclei divide and migrate through the mycelium. This would bring the rusts into line with the basidiomycetes in which Lehfeldt (19) and Buller (7) have studied the progressive diploidization of a gametophytic mycelium by the introduction of nuclei from another mycelium, by the division of these introduced nuclei, and by their migration throughout the mycelium.

SUMMARY

The gametophyte generation of *Puccinia graminis* on the barberry leaf produces spermogonia, receptive hyphae, and gametophytic accia.

When growing in young, tender leaf tissue hyphae push out freely between epidermal cells to both the upper and lower surfaces of the leaf and sometimes grow for a short distance along the surface. Where growing spermogonia at either leaf surface lift and stretch the epidermis and weaken it by tension, surface hyphae are formed in great abundance.

Barberry leaves rapidly become tough and resistant as the yapproach maturity and the outer epidermal walls become thickened. The surface hyphae already formed between epidermal cells die and disappear and new surface hyphae form with increasing difficulty or fail to develop altogether.

All stomata in the barberry leaf are in the lower epidermis. Hyphae in stomata are rare in young infections and those that do form are quickly killed by the closing of the stoma. A stoma, located where a spermogonium lifts and stretches the epidermis, may be forced wide open and soon fills with hyphae.

As the infection grows and the host tissues become hypertrophied and growing aecia add to the volume, the whole epidermis is under increased tension and stomata are easily opened. Wide-open stomata crowded with one to six hyphae become more and more common.

Under the conditions of the present experiments, fertilization commonly takes place at the upper surface through the spermogonia. In some instances this is the only possible means of entrance for spermatia. If fertilization is prevented, the spermogonia remain active as long as the fungus lives and continue to grow and broaden out and form fresh paraphyses, and the drop of spermogonial exudate (spermatia plus a liquid) is maintained. A day or two after fertilization, whether it takes place early or late, the formation of spermatia stops, the exudate dries, and the spermogonia die.

It is probable that fertilization also can occur by means of the stomatal hyphae of the lower surface, although this has not been observed.

The sporophyte spreads from the point or points of its origin by growth of new hyphae or by migration of nuclei through existing hyphae.

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WEEDS AS POSSIBLE CARRIERS OF LEAF ROLL AND RUGOSE MOSAIC OF POTATO¹

By T. P. DYKSTRA²

Assistant pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The host range of certain viruses, such as curly top of sugar beets (13),³ aster yellows (9), and cucumber mosaic (7), includes many different families of plants. It is known that certain viruses—for example, the latent virus of apparently healthy⁴ potatoes (3, 6, 11, 15)—may be masked in one host but show definite mottling in other hosts and a necrotic effect in still others. The symptoms of a virus may also appear as a mottling on one host and as at chlorosis when the virus is transferred to a different host. For example, Hoggan (4) found that when Johnson's tobacco virus no. 1 was transmitted to *Capsicum annuum* L. a chlorotic condition without mottling was produced.

Rugose mosaic and leaf roll are two of the most important virus diseases of potato (*Solanum tuberosum* L.) in Oregon. The former occurs in all parts of the State, whereas the latter is confined principally to the potato-growing areas east of the Cascade Mountains. In some sections of the State these diseases are difficult to control by roguing. It seems probable that weeds may serve as hosts of these and other virus diseases of potato and may be a source of infection when growing near potato fields.

In the summer of 1929, while inspecting potato fields in central Oregon that showed a large percentage of leaf roll, the writer discovered many plants of nightshade (*Solanum villosum* Mill.). Upon examination it was found that a number of these plants were infected with a disease resembling leaf roll. Infected plants were slightly dwarfed and their leaves were rolled, leathery, and chlorotic, whereas healthy plants in the same field were vigorous and had leaves of a normal green color.

A potato field showing about 50 percent rugose mosaic was found in the same section. Many of the plants displayed typical current-season symptoms of rugose mosaic, namely, necrosis and dropping of the leaves. Nightshade was likewise abundant in this field, but a thorough examination of several hundred plants did not reveal a single one that showed mottling or necrosis. It was therefore thought that this weed might not be a host of rugose mosaic; subsequent experiments have indicated, however, that it is.

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² The writer is indebted to E. S. Schultz, of the Division of Fruit and Vegetable Crops and Diseases, and H. P. Burss, of the Oregon Agricultural Experiment Station, for administrative help and for criticism of the manuscript, and to W. E. Lawrence and H. M. Gilkey, of the Oregon Agricultural Experiment Station, for ecological and taxonomic assistance.

³ Reference is made by number (italic) to Literature Cited, p. 31.

⁴ Apparently healthy potatoes are regarded as healthy in commercial practice, but they harbor in masked condition a virus disease which has been called "latent virus."

A study of the host range of the viruses that affect cultivated plants is of primary importance in formulating a program for their control. Since symptoms of the same virus may vary on different species of plants and even on different varieties of the same species, it is also essential to determine the symptomatology of these virus diseases on the various hosts. In order to learn more about host relationships of potato viruses, the experiments reported in this paper were begun in the fall of 1929.

REVIEW OF LITERATURE

The importance of weeds as hosts of virus diseases of potato has been mentioned in some publications (10, 12, 16, 17), but very few specific data have been presented bearing upon the relation of such hosts to the spread of these diseases to potatoes under natural field conditions.

Quanjer (10) states that tobacco (*Nicotiana tabacum* L.), *Atropa belladonna* L., *Datura stramonium* L., *Hyoscyamus niger* L., *Solanum nigrum* L., and *S. dulcamara* L., when infected by grafting or by means of aphids, are able to carry leaf roll although they do not show symptoms of it, as is indicated by the fact that the disease can be transmitted back to potatoes by grafting the vines with tops of such infection carriers. The species of aphids used and the percentage of infection secured are not given. The writer (2) has transmitted leaf roll by means of *Myzus persicae* (Sulz.) from potato to tomato, pepper, *Datura stramonium*, *D. tatula* L., *S. nigrum*⁶ and *S. dulcamara*.

Quanjer's crinkle, as pointed out by Johnson (7), is probably identical with Schultz and Folsom's (12) rugose mosaic. Quanjer claims to have transmitted crinkle by grafting to tomato (*Lycopersicon esculentum* Mill.), *Solanum nigrum*, *Datura stramonium*, *Atropa belladonna*, and *Hyoscyamus niger*. He states that the symptoms resembled those of potato, but makes no reference to return inoculation to potato.

Schultz and Folsom (12) found that the tomato is susceptible to mild mosaic and rugose mosaic. Young and Morris (17) transmitted rugose mosaic from potato to tomato. Fernow (3) secured but one disease when he transferred juice from diseased or healthy potatoes to other solanaceous plants. He referred to this as virus B. Blodgett (1) attempted to inoculate pepper plants with the virus of yellow-dwarf disease of potatoes and also with inoculum from apparently healthy potatoes. All the peppers inoculated from potato, either from the yellow-dwarf tubers or from the healthy tubers, showed the same type of symptom, namely, a severe necrosis of the leaves and stems. He does not state whether return inoculations to potato were made from peppers inoculated with yellow dwarf. Johnson (5) found that "healthy" potato virus and vein banding from tobacco, when inoculated into pepper plants, sometimes produced chlorotic rings and necrosis, followed by defoliation. These symptoms never developed from the "healthy" potato virus alone, and the vein-banding virus was not recovered when return inoculations were made to tobacco. Johnson (6) was the first to demonstrate the presence of one or several viruses in practically all so-called healthy commercial American

⁶ A later and more accurate determination of this species establishes it as *Solanum villosum*. The two species are decidedly similar, but *S. villosum* is the more hairy of the two and its calyx lobes are broad and triangular and united for half their length, whereas *S. nigrum* is glabrous and its calyx lobes are narrow and separate almost to the base.

varieties of potatoes. Valteau (15) reported similar results. Schultz (11) found that when certain apparently healthy potato varieties were tuber-grafted with each other and with seedlings, necrotic streaks developed on some of the varieties and seedlings.

Johnson (5) refers to a disease occurring naturally on tobacco as "vein banding," on account of the narrow bands of dark-green tissue that appear along the veins of the leaves of affected plants. He transferred this virus from tobacco to tomato, petunia (*Petunia hybrida* Vilm.), and species of *Physalis*. Valteau and Johnson (16) transmitted the vein-banding virus from tobacco to apparently healthy young Irish Cobbler potato plants and produced typical rugose-mosaic symptoms. When the vein-banding virus was transmitted to potato seedlings free from the latent virus of healthy potatoes, it caused a disease characterized by nearly normal color, faint mottling, rugose-ness, and only slight distortion of the leaves.

Smith (14) found that when juice of mosaic-affected potatoes was pricked into healthy tobacco plants a ring spot developed. Transmission by the aphid *Myzus persicae* of a virus from mosaic potatoes to tobacco resulted in vein banding. When juice from healthy potatoes was transmitted no symptoms appeared. Koch (8) found that the rugose mosaic of potato consists of two distinct viruses. One is easily transmitted by aphids; the other, the "mottle," or latent virus of apparently healthy potato, can be transmitted mechanically but not by aphids. The aphid-transmitted or vein-banding virus, when transferred to tobacco, caused only a clearing of the veins. When transmitted mechanically, the combined rugose-mosaic viruses (vein-banding virus plus latent virus) produced spot necrosis on tobacco.

MATERIALS AND METHODS

The following plants were grown from seed and studied as hosts of virus diseases of potato: jimsonweed (*Datura stramonium* and *D. tatula*), nightshade (*Solanum villosum*), bitter nightshade (*S. dulcamara*), groundcherry (*Physalis* sp.), tomato (*Lycopersicon esculentum*), redpepper (*Capsicum annuum* L.), and petunia (*Petunia hybrida*).

Tubers known to be infected with rugose mosaic or leaf roll and also those from apparently healthy potatoes were planted in pots in the greenhouse. When potatoes infected with rugose mosaic or so-called healthy potatoes served as the source of inoculum, the leaf-mutilation method was used; that is, the leaves from the diseased plants were macerated and the extracted juice was rubbed into the leaves of healthy plants. Previous studies had shown that the incubation period of rugose mosaic is from 3 to 4 weeks; therefore 6 weeks after the plants were inoculated, juice was extracted from their leaves and rubbed into the leaves of a series of healthy potato plants. Potato plants containing the latent virus will manifest necrotic spots on the leaves if they are inoculated by leaf mutilation with the vein-banding virus (fig. 1).

In the spring of 1931, aphids (*Myzus persicae*) were colonized on a Burbank potato plant affected with rugose mosaic and were then transferred to three Marglobe tomato plants. No symptoms developed except a faint clearing of the veins, nor did these tomato plants appear to be stunted in their growth. This species of aphid

does not transfer the latent virus of apparently healthy potatoes, as was determined in tests including nearly 100 plants; it transmits only the vein-banding component of the rugose mosaic. This aphid-transmitted virus has been transferred mechanically by leaf mutilation from tomato to apparently healthy potatoes, potato seedlings, and other solanaceous plants. Valleau and Johnson (16) found this

virus occurring naturally on tobacco and referred to the disease as vein banding. In the present paper the term "vein banding" is used for the aphid-transmitted component of rugose mosaic.

The aphid-transmission method was used to transmit leaf roll, since it cannot be transmitted by leaf mutilation. Aphids (*Myzus persicae*) were colonized under muslin-covered insect cages on potato plants infected with leaf roll and left there for 2 weeks or more. About 20 of the aphids were then transferred to each of the plants that were to be inoculated and left there for 6 days. The cages were then removed and the plants were fumigated to kill the aphids. About 20 to 25 days after inoculation, when symptoms of leaf roll appeared, non-viruliferous aphids were colonized on these plants and later transferred to young healthy potato plants, which were also fumigated after 6 days.



FIGURE 1 - American Giant (Pride of Wisconsin) potato plant containing the latent virus, inoculated by leaf mutilation with vein-banding mosaic from an infected potato plant, shows necrotic spots on leaves

The greenhouse was fumigated frequently. Numerous potato plants and other solanaceous plants were growing in the greenhouse, but not a single case of leaf roll developed on any of the uninoculated plants.

The aphids used were obtained from carnation plants growing in one of the greenhouses and were colonized on cabbage plants and egg-

plants growing under muslin-covered insect cages. Occasionally these aphids were transferred to potato plants to determine whether the aphids were free from the leaf-roll virus; in all cases the potato plants remained healthy.

SYMPTOMS OF RUGOSE MOSAIC, VEIN BANDING, AND LEAF ROLL ON VARIOUS PLANTS

SYMPTOMS ON JIMSONWEED

Two species of jimsonweed (*Datura stramonium* and *D. tatula*) developed a pronounced mottling when inoculated by leaf mutilation with juice from apparently healthy potato plants or from potato plants affected with rugose mosaic. The mottling was characterized by large yellowish-green blotches on the leaves.

Attempts were made to transmit the rugose mosaic by leaf mutilation from either species of *Datura* inoculated with this disease to potato plants, but all results were negative (table 1).

TABLE 1.—Cross inoculations with rugose mosaic from potato by leaf mutilation

Species tested	Plants inoculated with rugose mosaic from potato		Return inoculations to potato plants		
	Total	Infected	Total	Infections	Notes
	Number	Number	Number	Number	
<i>Solanum villosum</i>	10	7	15	13	Latent-virus mottling
<i>Physalis</i> sp.	10	6	10	8	Latent-virus mottling and some rugosity
<i>Petunia hybrida</i>	10	7	(*)	—	—
<i>Lycopersicon esculentum</i>	25	25	30	27	—
<i>Datura tatula</i>	10	0	10	0	Latent-virus mottling
<i>Datura stramonium</i>	10	0	10	0	Do
<i>Capsicum annuum</i>	15	0	10	0	Necrosis, like that caused by the latent virus
<i>Solanum dulcamara</i>	10	0	10	0	No symptoms

* No return inoculations to potato

The vein-banding mosaic failed to produce any symptoms when introduced into jimsonweed, nor could the virus be recovered by making return inoculations into young potato plants (table 2). Valleau and Johnson (16) reported similar results.

TABLE 2. Cross inoculations of aphid-transmitted vein-banding component of rugose mosaic by means of leaf mutilation

Species tested	Plants inoculated with vein banding from tomato		Return inoculations to potato plants	
	Total	Infected	Total	Infections
	Number	Number	Number	Number
<i>Solanum tuberosum</i> (seedling)	20	17	(*)	—
<i>Solanum tuberosum</i>	20	18	(*)	—
<i>Solanum villosum</i>	10	10	6	6
<i>Physalis</i> sp.	10	7	10	7
<i>Lycopersicon esculentum</i>	15	15	(*)	—
<i>Datura stramonium</i>	10	0	6	0
<i>Petunia hybrida</i>	10	8	6	6

* No return inoculations to potato.

Datura does not appear to be a host of the vein-banding component of rugose mosaic, but it is very susceptible to the latent virus of apparently healthy potatoes. In the summer of 1930, 6 plants of *Datura stramonium* and 6 of *D. tatula* were planted in a potato field, and every one of these weeds developed a pronounced mottling as a result of natural transmission of the latent virus from apparently healthy potato plants. It was not determined whether this transmission was due to insect transfer or to other means, such as the processes of cultivation or mechanical contact of the leaves caused by the wind.

Leaf roll was transmitted by aphids from potato to *Datura stramonium* and *D. tatula* and from these weeds to potato (table 3). On the two species of *Datura*, leaf roll was characterized by a distinct chlorosis



FIGURE 2.—*Datura stramonium* infected with leaf roll through the agency of aphids (*Myzus persicae*) that were transferred from an infected potato plant, shows chlorosis and rolling of leaves

and rolling of the leaves, which became leathery as the disease progressed (fig. 2).

TABLE 3.—Cross inoculations with leaf roll by means of aphids (*Myzus persicae*)

Source of inoculum	Species tested	Plants inoculated with leaf roll		Return inoculations to potato	
		Total	Infected	Total	Infections
		Number	Number	Number	Number
Potato	<i>Datura tatula</i>	5	5	4	3
	<i>Datura stramonium</i>	10	9	10	7
	<i>Solanum villosum</i>	12	10	7	6
	<i>Solanum dulcamara</i>	9	(*)	6	5
	<i>Capiscum annuum</i>	5	4	8	b 3
	<i>Lycopersicon esculentum</i>	15	13	9	7
	<i>Physalis</i> sp.	10	0	10	0
	<i>Solanum tuberosum</i>	11	10		
	<i>Lycopersicon esculentum</i>	4	3	(c)	
	<i>Solanum villosum</i>	6	5	(c)	
Tomato	<i>Datura stramonium</i>	2	2	(c)	
	<i>Lycopersicon esculentum</i>	6	5	(c)	

* Apparently symptomless carrier of leaf roll.

b Symptoms not typical of leaf roll.

c No return inoculations to potato.

SYMPTOMS ON NIGHTSHADE

Nightshade (*Solanum villosum*) developed a pronounced mottling when inoculated with juice from apparently healthy potato plants or from those infected with rugose mosaic. When infected plants were growing in the greenhouse or under muslin-covered insect cages, the mottling was quite distinct. In the summer of 1930 three nightshade plants infected with rugose mosaic were planted outdoors, but during the summer the symptoms were completely masked and it was impossible to recognize the disease on the plants. However, rugose mosaic was readily transmitted from infected *Solanum villosum* to potato by leaf mutilation (table 1).

Vein-banding virus alone caused a faint vein banding in some of the nightshade leaves, but the symptoms were generally difficult

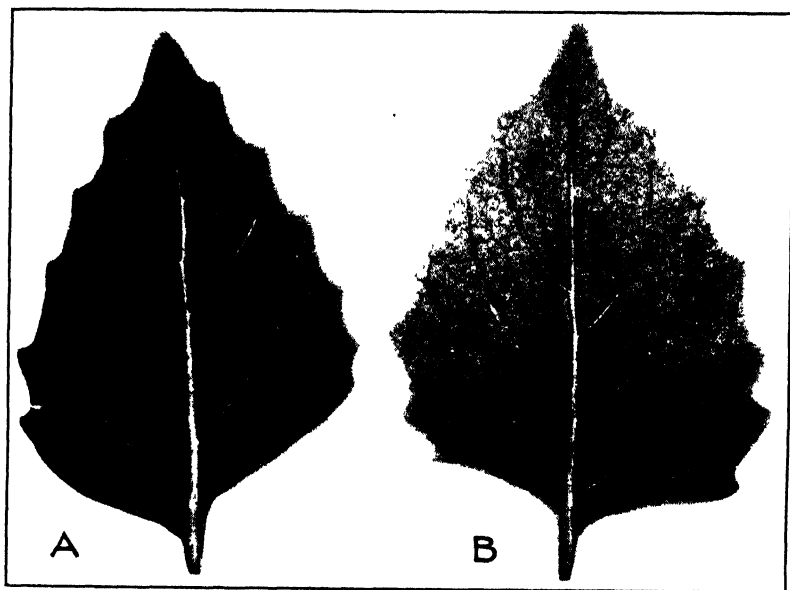


FIGURE 3 -Leaves of *Solanum villosum*. A, Healthy leaf. B, leaf from plant inoculated by leaf mutilation with vein-banding mosaic from an infected tomato plant, showing dark bands along veins characteristic of the disease

to detect (fig. 3). The virus from such infected weeds can be readily transferred by leaf mutilation to apparently healthy potatoes, causing typical rugose-mosaic symptoms (table 2).

The leaf-roll virus was readily transmitted from potato to nightshade and returned to potato by *Myzus persicae*. The symptoms on nightshade were distinct chlorosis and rolled leaves (fig. 4). The disease was first apparent in some of the younger leaves, which became leathery as the disease progressed; finally all leaves of the plant showed leaf-roll symptoms (table 3).

SYMPTOMS ON BITTER NIGHTSHADE

Bitter-nightshade plants (*Solanum dulcamara*) were inoculated with juice from healthy potato plants and also from potato plants affected with rugose mosaic, but no symptoms of any kind developed, nor

was it possible to recover the rugose-mosaic virus when return inoculations were made to potato (table 1).

When leaf roll is transferred by aphids from potato to bitter-nightshade plants the disease may show very strikingly. One plant to which the disease was transmitted ceased growing and became very much stunted, although it remained alive. The leaves became chlorotic and stiff. Other bitter-nightshade plants inoculated at the same time in a similar manner, although carrying the virus, appeared healthy. Leaf roll was transmitted from both types of plant back to potato (table 3).

SYMPTOMS ON TOMATO

When a tomato plant (*Lycopersicon esculentum*) was inoculated with juice from an apparently healthy potato plant, a faint mottling of pale-green areas developed which was distributed irregularly



FIGURE 4.--*Solanum rillosum* infected with leaf roll through the agency of aphids (*Myzus persicae*) that were transferred from an infected potato plant, shows chlorosis and rolling of leaves.

over the surface of the leaf. Inoculations from potatoes infected with rugose mosaic generally caused a necrosis of the leaves. This disease was readily transmitted from infected tomato plants back to potato. In some cases, instead of a necrosis, mottling developed that was indistinguishable from the type caused by the latent virus. Three tomato plants showing such symptoms were grown in the field under cages during the summer of 1930, but they did not develop necrosis. When juice from these plants was inoculated into potato, however, typical symptoms of rugose mosaic resulted (table 1).

In the spring of 1931, aphids (*Myzus persicae*) from a Burbank potato plant infected with rugose mosaic were transferred to three Marglobe tomato plants. Six weeks after inoculation the tomato plants appeared healthy except for a faint clearing of the veins. Return inoculations by leaf mutilation from each of the three plants into potato plants already carrying the latent virus resulted in typical rugose-mosaic symptoms. This indicated that the aphids had

transmitted the vein-banding component of rugose-mosaic virus to each of the three tomato plants, causing only a slight clearing of the veins and apparently not interfering with normal growth. As previously indicated, in the transfer of leaf roll from potato to solanaceous weeds by *M. persicae* where only leaf roll and not the latent virus was transmitted, this species of aphid is unable to transmit the latent virus.

When the vein-banding virus was transferred by leaf mutilation from tomato to potato seedlings and to plants raised from tubers from second-year seedlings, only an interveinal type of mottling developed, without necrosis or rugosity. These plants remained vigorous for a long time and their growth did not appear to be appreciably checked by the disease. However, when potato seedlings were inoculated by leaf mutilation with the latent virus of apparently healthy potatoes, a severe necrosis of leaves and stems developed, causing the death of the plants 16 days after inoculation (fig. 5).

Leaf roll was also readily transferred from potato to tomato by aphids (table 3). The symptoms were not very distinct. The leaves showed practically no rolling but became rigid and somewhat leathery; no stunting of the plant was observed.

Two tomato plants infected with leaf roll were inoculated with tomato mosaic, and although the latent virus was not present, the combination of the leaf-roll virus and tomato mosaic developed typical streak.

Leaf roll was readily transmitted from tomato to potato by means of aphids (fig. 6); it has also been transferred from tomato to jimsonweed and nightshade in the same way.



FIGURE 5. Potato seedling showing necrosis on leaflets 12 days after inoculation by leaf mutilation with the latent virus from an apparently healthy potato plant.

SYMPTOMS ON GROUNDCHERRY

When the common groundcherry (*Physalis* sp.) was inoculated by leaf mutilation with the latent virus from an apparently healthy potato plant, a faint mottling developed. Inoculation with virus from potatoes infected with rugose mosaic caused a more pronounced mottling and some rugosity, but apparently no necrosis (fig. 7).

Rugose mosaic was also readily transferred from infected *Physalis* to potato (table 1).

When groundcherry plants were inoculated with the vein-banding virus, no symptoms developed; 5 weeks after inoculation, however,



FIGURE 6 American Grant (Pride of Wisconsin) potato plant infected with leaf roll through the agency of aphids (*Myzus persicae*) that were transferred from an infected tomato plant

when inoculum from a number of the inoculated plants was transferred to potato plants infected with latent virus, typical rugose-mosaic symptoms developed.

No symptoms were evident after *Physalis* plants were inoculated by means of aphids with leaf roll from potato, nor could the disease be retransferred from such inoculated plants to potato (table 3).

SYMPTOMS ON REDPEPPER

When young redpepper plants (*Capsicum annuum*) were inoculated by leaf mutilation with the latent virus from apparently healthy potato plants or the juice from those infected with rugose mosaic, severe necrosis and leaf dropping resulted (table 1). Apparently there were no differences in the symptoms caused by the two viruses. Transfers to potato of juice from peppers inoculated with rugose



FIGURE 7 Groundcherry (*Physalis* sp.) showing mottling and rugosity of the leaves produced by inoculating the plant by leaf mutilation with juice from a potato plant infected with rugose mosaic.

mosaic, however, failed to produce any symptoms. No inoculations were made with the vein-banding virus alone.

During the summer of 1930 eight pepper plants were planted in a potato field, but no necrosis or leaf dropping due to probable natural transmission from the potatoes developed.

Attempts were made to transfer leaf roll by means of aphids from potato to pepper. Some rolling and chlorosis developed in the affected plants. When the disease was transferred back to potato some rolling developed, but the symptoms were not typical of leaf roll. Further testing is needed before pepper can be classified as a host of leaf roll (table 3).

SYMPTOMS ON PETUNIA

Plants of petunia (*Petunia hybrida*) were inoculated by leaf mutilation with rugose mosaic from potatoes (fig. 8). Of the 10 plants inoculated, only 1 developed severe necrosis; the other 9 showed no symptoms of disease. No return inoculations from these plants to potato were made. Later inoculations with rugose mosaic have resulted in a mottling or a necrosis of the petunia leaves in a very large percentage of the plants inoculated.

When the vein-banding virus was inoculated into petunia, a faint clearing of the veins developed. After the plants became older these



FIGURE 8 Petunia showing necrosis on some of the leaves as a result of inoculation by leaf mutilation with juice from a potato plant infected with rugose mosaic

symptoms disappeared. Return inoculations from these plants to potatoes carrying the latent virus developed typical rugose-mosaic symptoms.

No attempt was made to transmit leaf roll to this host.

TRANSMISSION OF LEAF ROLL AND RUGOSE MOSAIC BY INSECTS OCCURRING ON POTATO PLANTS

It was considered important to determine whether insects that feed on potato plants under natural conditions are able to transmit virus diseases from infected weeds to potato. Accordingly two large muslin-covered insect cages were built. In the larger cage the following plants infected with leaf roll were interplanted among 85 healthy potato plants: *Solanum rillosum*, 6 plants; *Datura stramonium*, 4 plants; tomato, 1 plant. Insects collected from a field of healthy

potatoes at the Oregon Agricultural Experiment Station at Corvallis, Oreg., were introduced into the cage. During the growing season 11 percent of the potato plants developed current-season symptoms of leaf roll. At digging time the progenies of all the potato plants were saved, and in the spring of 1931 they were planted in the field. Of the 433 tubers planted, 355 (82 percent) developed into plants showing typical tuber-perpetuated leaf-roll symptoms.

In the smaller cage, 6 *Solanum villosum* plants and 2 tomato plants, all of which were infected with rugose mosaic, were planted among 52 healthy potato plants. Miscellaneous insects, collected from the same field of healthy potatoes as were the insects used to transmit leaf roll, were introduced into the cage in order to determine whether they would spread rugose mosaic from the weeds to the potato plants.

During the growing season 10 percent of the potato plants developed current-season symptoms of rugose mosaic. The progenies from all the plants were saved and planted in the field in 1931. Of the 157 tubers planted, 84 (53 percent) developed typical tuber-perpetuated rugose mosaic; but in addition to this, 9 plants (6 percent) developed tuber-perpetuated leaf-roll symptoms. Since the smaller cage did not have a double door like that built in the larger one, and since the two cages adjoined each other, there is a possibility that some insects from the larger cage were accidentally introduced into the smaller one. No leaf roll was present in the field from which the insects were collected, nor were any current-season symptoms of leaf roll evident in the plants of the smaller cage during 1930.

Specimens of the insects used in these transmission experiments were sent for identification to H. Morrison, of the Bureau of Entomology, United States Department of Agriculture. The following species were identified: *Myzus persicae*, *Illinoia solanifolii* (Ashm.), *Epitrix subcrinita* Lec., *Sciara* sp., *Chironomus* sp., *Empoasca* sp., cicadellid species, *Nabis alternatus* Parsh., *Lygus pratensis* (L.), and *Phlaenus spumarius* Fall.

Experiments are now in progress to determine the ability of each of these insects to transmit the virus diseases of the potato.

ATTEMPTS TO TRANSMIT THE LATENT VIRUS BY MEANS OF INSECTS

It is of interest to know whether or not the latent virus of apparently healthy potatoes is readily transmitted by insects. This information is of importance in determining whether promising new potato seedlings should be grown in isolation from apparently healthy potatoes in order to keep them from becoming infected with the latent virus. This knowledge is also valuable in the testing of potato seedlings for resistance to virus diseases. The symptoms of some of these diseases on potatoes free from the latent virus are very mild, and this may erroneously be interpreted as resistance or tolerance of the potatoes to virus diseases.

In order to secure some data on insect transmission of the latent virus, five different species of insects occurring on potato, namely, western potato flea beetle (*Epitrix subcrinita*), leaf hopper (*Empoasca* sp.), tarnished plant bug (*Lygus pratensis*), spittle bug (*Phlaenus spumarius*), and *Nabis alternatus*, were collected from a field of healthy potatoes.

In the late summer of 1931, 8 to 10 insects from each of these 5 species were transferred to 5 separate lots, respectively, consisting of 12 individually caged jimsonweed plants. The insects were left on the plants for 7 days; the plants were then fumigated to kill the insects, and the cages were removed. In all cases the jimsonweeds remained normal, with the exception of those to which the spittle bug (*Philaenus spumarius*) had been transferred. These 12 plants developed large yellow blotches on the leaves about 12 days after the insects had been transferred to them. The type of mottling was not quite typical of that caused by the latent virus.

To determine whether the mottling was due to the latent virus or to an enzyme or a toxin injected by the insect, this same species of insect was collected from an alfalfa field and transferred to 10 tomato plants and 10 jimsonweeds. No symptoms developed on the tomato plants, but some blotches were evident on the jimsonweeds about 12 days after the spittle bugs had been transferred to them.

Additional spittle bugs (*Philaenus spumarius*) were collected from a potato field and transferred to 10 tomato plants, none of which developed any symptoms.

No symptoms developed when 10 tomato plants were inoculated by leaf mutilation with juice from jimsonweeds that showed yellow blotches on the leaves after spittle bugs collected from potato plants had fed on them.

These experiments will have to be repeated on a larger scale, and other insects occurring on potato will have to be tested, before any final conclusions can be drawn; but the results secured so far do not indicate that the latent virus of apparently healthy potatoes is readily transmitted by insects.

DISCUSSION AND CONCLUSIONS

It has been demonstrated that certain weeds growing in or near potato fields may, under natural conditions, become infected with potato-virus diseases. Insects naturally occurring on potato plants are able to transmit leaf roll and rugose mosaic from weeds to potato.

When solanaceous weeds become naturally infected with rugose mosaic, only the vein-banding virus may be present; the symptoms caused by this virus on weeds are very difficult to detect.

The fact that vein banding caused only mild mottling on potatoes free from the latent virus, in contrast to the severe disease it develops on apparently healthy potatoes carrying the latent virus, suggests that the effects of certain potato-virus diseases, namely, vein banding and leaf roll, may be greatly intensified by the presence of the latent virus.

Potato seedlings that were inoculated with leaf roll remained alive and vigorous in pots in the greenhouse considerably longer than did apparently healthy potato plants carrying the latent virus that were inoculated with leaf roll.

Weeds infected with leaf roll generally show chlorosis, rolling of the leaves, and a tendency to be leathery.

SUMMARY

Rugose mosaic was transmitted by leaf mutilation from potato to *Solanum villosum*, *Physalis* sp., tomato, and petunia.

Aphids (*Myzus persicae*) did not transmit the latent virus from apparently healthy potatoes to other solanaceous plants.

From potatoes infected with rugose mosaic, *Myzus persicae* transmitted to tomatoes only the vein-banding component of the virus complex.

The vein-banding virus alone causes very faint symptoms in susceptible host plants, characterized in soft-leaved plants by a banding of the veins.

The vein-banding virus when inoculated into apparently healthy potato plants (carrying the latent virus) causes typical rugose-mosaic symptoms; when inoculated into potato seedlings free from the latent virus, vein banding causes only a faint type of mottling.

Leaf roll was transmitted by *Myzus persicae* from potato to *Solanum villosum*, *S. dulcamara*, *Datura stramonium*, *D. tatula*, and *Lycopersicon esculentum*.

Insects naturally occurring on potato can transmit leaf roll and rugose mosaic from infected weeds to potato.

Five different species of insects naturally occurring on potato failed to transmit the latent virus of apparently healthy potatoes to jimson-weed.

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EFFECTS OF PLANT SPACING AND IRRIGATION ON NUMBER OF LOCKS IN COTTON BOLLS¹

By A. R. LEDING, *chief scientific aid*, and L. R. LYTTON, *assistant scientific aid*, Division of Cotton, Rubber, and Other Tropical Plants, Bureau of Plant Industry, United States Department of Agriculture²

INTRODUCTION

Practically all the bolls produced by American upland varieties of cotton (*Gossypium hirsutum* L.) have either four or five locks or carpels. Some varieties produce a greater proportion of 5-lock bolls than others, and there is sometimes considerable variation among individual plants of the same variety. The factors that determine the ratio of the 4-lock to the 5-lock bolls are obscure, but are generally considered to be heritable. It is clear, however, that the proportion is materially affected by the conditions under which the plants are grown, such as infertile or alkaline soil; an insufficient supply of moisture; extremes of temperature, sometimes accompanied by a reduction of atmospheric humidity; crowding of the plants in the row; the presence of weeds; and any other conditions that limit optimum development.

When grown under good conditions the Acala variety of upland cotton produces from 75 to 85 percent of 5-lock bolls. The majority of these are set during the middle part of the growing season, after the plants have attained some size but before the general slackening of growth toward the end of the season. In the earlier part of the season, and again toward the latter part, the proportion of 4-lock bolls is often increased and may equal and sometimes exceed the number of 5-lock bolls. Accordingly, it is frequently found that the 4-lock bolls are borne at the inner nodes of the lower fruiting branches and on the outer nodes of the upper branches, although this habit is not constant. Vegetative branches, as a rule, produce a higher percentage of 4-lock bolls than do the fruiting branches.

The number of locks is probably determined very early in the embryonic stage of the boll. At this stage, apparently, the heritable factor that normally determines the number of locks is influenced by physiological conditions in the plant, induced by the conditions under which the plant has been growing. Just when or how these effects are brought about is beyond the scope of this paper. The evident effects of certain environmental conditions on the ratio of 4-lock to 5-lock bolls have been noted, however, and are here discussed.

REVIEW OF LITERATURE

In the study of the morphology of the cotton plant the lock number has received considerably less attention than some other charac-

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ters, and the literature on the subject is limited. The effect of changed growth conditions was noted in Texas in 1908 by the late Rowland M. Meade, as reported by Cook (3),³ in a planting of Kekchi cotton, a variety native to Guatemala, which normally has a large proportion of 5-lock bolls. Plants from newly imported seed of this variety and from seed of an acclimatized strain, grown near together, showed striking differences, including a greatly decreased proportion of 5-lock bolls in the plants grown from the newly imported seed.

Cook further discussed the effect of changed environment on this character of the cotton plant in 1913 (4). Data are given from experimental plantings of Kekchi cotton in 10 localities in California, showing different proportions of lock numbers in the bolls, with the general observation that "drought or other adverse conditions that reduce the crop also diminish the proportion of 5-locked bolls."

In comparing plants spaced at about 2 feet with spacings of 6 to 8 inches in the row at San Antonio, Tex., in 1914, Meade (9, p. 13) found that "the range in percentage of 5-locked bolls in wide-spaced rows was from 47 to 72, with an average of 56, while in single-stalk rows the range was from 43 to 61 percent, with an average of 46 percent."

Ballard and Simpson (1), working at San Antonio, found on cotton plants whose growth had been retarded by Johnson grass a consistently higher percentage of 4-lock bolls than on plants free from grass in another part of the field.

Although it is generally known that some varieties of upland cotton produce more 5-lock bolls than others, comparatively little has been published on the heritability of the lock-number character in this or other species of cotton. Working in Arizona with Pima Egyptian, normally a 3-lock variety, Kearney (8) found that in two selections having relatively high percentages of 4-lock bolls this character was inherited by the progenies. A cross between these strains, in the fourth hybrid generation in 1929, produced 9.1 percent of 4-lock bolls, in contrast with four normally 3-lock inbred strains, which averaged only 3.4 percent of 4-lock bolls.⁴

Harland (7, p. 159), working in the West Indies with sea-island cotton, which is normally 3 lock, found that "by selection the percentage of 4-locked bolls has been increased from 20 (the value for ordinary sea island) to 60 or 70." Dunlavy (6, p. 445) states:

Previous work with Acala cotton tends to indicate that the five-lock boll is from ten to fifteen percent larger than the "four" and that the relationship between fours and fives is a heritable character, though greatly influenced by environmental conditions.

There is a well-established preference among farmers for a variety or strain of cotton which produces a large proportion of 5-lock bolls. Since the 5-lock bolls are larger they are supposed to produce more cotton, and in most cases they do, though varieties having a large proportion of 4-lock bolls may yield well. Tyler (10) and Dunlavy (5) have reported comparative weights of 4-lock and 5-lock bolls in several varieties of upland cotton.

³ Reference is made by number (italics) to Literature Cited, p. 52.

⁴ Unpublished data.

EFFECT OF PLANT SPACING ON NUMBER OF LOCKS

EXPERIMENTS IN 1926

The 1926 cotton-spacing experiments at the United States Acclimatization Field Station, State College, N.Mex., consisted of comparisons, in alternate 4-row blocks, of 2 plants per foot, a so-called "blocked-out" spacing, and unthinned plants, with a standard spacing of 12 inches, or 1 plant to each foot of row. In the 2-plant spacing, the plants were thinned to 2 per hill, with the hills approximately 1 foot apart. In the blocked-out method, the plants were chopped out with an 8-inch hoe, leaving small blocks of plants approximately 8 inches long separated by intervals of similar length. In the unthinned rows the plants grew under crowded conditions, with sometimes as many as 7 or 8 plants per foot, in contrast with the 12-inch-spaced rows, where the plants approximated 1 per foot.

In the fall of the year, when small samples of 4-lock and 5-lock bolls were being picked for laboratory use, it was noted that the requisite number of 4-lock bolls was more readily obtained from the unthinned plants than from the plants spaced at 12 inches. An examination was therefore made of 10-foot sections of the inside rows of each 4-row block in the experiment, and the 4-lock and 5-lock bolls were counted. A small but significant difference was found in the percentages of 4-lock bolls in the two spacings, there being 38.52 ± 1.17 percent in the unthinned plants and 32.66 ± 1.16 percent in the 12-inch-spaced plants, or a difference of 5.86 ± 1.65 percent. A similar tendency appeared in the comparison of one and two plants per foot. Though the percentage differences were small, they indicated that the spacing of the plants had an effect on the proportion of 4-lock bolls produced.

EXPERIMENTS IN 1927

In 1927 somewhat more comprehensive data were obtained on the effect of spacing on the number of locks in the boll; the effect of the 2-plant and blocked-out spacings was studied also. In the 2-plant test, three 4-row blocks of this spacing alternated with three 4-row blocks in which the spacing was 1 plant to 12 inches. Similarly, in the blocked-out test, three 4-row blocks of blocked-out plants alternated with 3 blocks of the 12-inch spacing, but in the unthinned test there were only two 4-row blocks of each spacing. The data were obtained from 300 bolls on scattered small groups of contiguous plants selected at random in the inner rows of each 4-row block. In both the 2-plant and blocked-out tests, therefore, 900 bolls were examined for each method of spacing; in the unthinned test only 600 bolls were examined for each spacing.

The number of 4-lock and 5-lock bolls and the percentage of 4-lock bolls by separate blocks and by totals, with the average number of plants per foot are shown in table 1.

No difference was found in the proportion of 4-lock bolls in the test of one and two plants per foot, both spacings showing 21.88 ± 0.93 percent. The least difference in the average number of plants per foot occurred in this test, the 1-plant spacing averaging 1.15 plants per foot and the 2-plant spacing averaging 1.62. There was apparently no crowding effect in this test.

TABLE 1.—*Number of 4-lock and 5-lock bolls, percentage of 4-lock bolls, and average number of plants per row foot in spacing experiments, 1927*

Test no, block no, and treatment	Average number of plants per row foot	4-lock bolls	5-lock bolls	4-lock bolls
		<i>Number</i>	<i>Number</i>	<i>Percent</i>
Test 1				
Block 1 (1 plant to 12 inches)	1 03	65	235	21 67±1 60
Block 2 (blocked out)	2 62	95	205	31 67±1 81
Block 3 (1 plant to 12 inches)	.99	78	222	26 00±1 71
Block 4 (blocked out)	2 56	122	178	40 67±1 91
Block 5 (1 plant to 12 inches)	.98	73	227	24 33±1 67
Block 6 (blocked out)	2 40	91	209	30 33±1 79
Total (1 plant to 12 inches)	1 00	216	684	24 00±. 96
Total (blocked out)	2 53	308	592	34 22±1 07
Test 2				
Block 1 (1 plant to 12 inches)	1 18	73	227	24 33±1 67
Block 2 (2 plants to 12 inches)	1 68	62	238	20 67±1 58
Block 3 (1 plant to 12 inches)	1 13	62	238	20 67±1 58
Block 4 (2 plants to 12 inches)	1 61	67	233	22 33±1 62
Block 5 (1 plant to 12 inches)	1 13	62	238	20 67±1 58
Block 6 (2 plants to 12 inches)	1 58	68	232	22 67±1 63
Total (1 plant to 12 inches)	1 15	197	703	21 88±. 93
Total (2 plants to 12 inches)	1 62	197	703	21 88±. 93
Test 3				
Block 1 (1 plant to 12 inches)	1 43	80	220	26 67±1 72
Block 2 (unthinned)	5 85	138	162	46 00±1 94
Block 3 (1 plant to 12 inches)	1 41	66	244	22 00±1 61
Block 4 (unthinned)	1 71	109	191	36 33±1 87
Total (1 plant to 12 inches)	1 44	146	454	24 33±1 18
Total (unthinned)	5 28	247	353	41 17±1 31

In the blocked-out test, the 12-inch-spaced plants produced 24 ± 0.96 percent of 4-lock bolls and the blocked-out plants 34.22 ± 1.07 percent, a difference of 10.22 ± 1.44 percent. The average number of plants per foot was 1.0 and 2.53 for the wider and closer spacings, respectively. The effect of closer spacing was apparent here.

In the unthinned plants there was an average of 41.17 ± 1.31 percent of 4-lock bolls, while the compared blocks of 12-inch-spaced plants produced 24.33 ± 1.18 percent, a difference of 16.84 ± 1.76 percent. The average number of plants per foot in the unthinned blocks was 5.28 and in the 12-inch blocks 1.44. The effect of crowding on the production of 4-lock bolls appeared very definite in this test.

EXPERIMENTS IN 1928

The study of spacing effect was continued in the 1928 tests, the same method for obtaining the data being used as in the previous season. The data are presented in table 2.

The greatest differences in the numbers of 4-lock bolls produced were again found where the greatest differences in plant spacing existed. In the unthinned blocks the plants averaged 5.60 per foot, while in the 12-inch blocks with which they were compared there were but 1.01 plants per foot. A greater difference was found between the percentages of 4-lock bolls in these spacings than in 1927, the unthinned plants having 39.11 ± 1.10 percent and the 12-inch-spaced plants 18.22 ± 0.87 percent, a difference of 20.89 ± 1.40 percent. The relation of the percentages in the separate blocks to the number of plants per foot, however, was not as uniform as in 1927. In test 3 there was

a difference of 23 per cent between blocks 2 and 4, although the difference in the number of plants per foot was relatively small. On the other hand, block 6, where the number of plants was substantially greater than in either block 2 or block 4, had fewer 4-lock bolls than the former, the difference being approximately 20 percent, and but a few more 4-lock bolls than the latter, the difference in this case being but a little over 2 percent.

TABLE 2 - *Number of 4-lock and 5-lock bolls, percentage of 4-lock bolls, and average number of plants per row foot in spacing experiments, 1928*

Test no., block no., and treatment	Average number of plants per row foot	4-lock bolls	4-lock bolls	4-lock bolls
		Number	Number	Per cent
Test 1				
Block 1 (1 plant to 12 inches)	0.97	65	235	21.67 ± 1.60
Block 2 (blocked out)	3.46	87	213	29.00 ± 1.76
Block 3 (1 plant to 12 inches)	1.09	37	263	12.33 ± 1.28
Block 4 (blocked out)	3.13	100	200	33.33 ± 1.84
Block 5 (1 plant to 12 inches)	1.05	45	255	15.00 ± 1.39
Block 6 (blocked out)	3.35	86	214	28.67 ± 1.86
Total (1 plant to 12 inches)	1.04	147	753	16.33 ± .83
Total (blocked out)	3.31	273	627	30.33 ± 1.03
Test 2				
Block 1 (1 plant to 12 inches)	1.08	52	248	17.33 ± 1.47
Block 2 (2 plants to 12 inches)	1.84	69	251	23.00 ± 1.64
Block 3 (1 plant to 12 inches)	1.09	37	263	12.33 ± 1.28
Block 4 (2 plants to 12 inches)	1.90	64	236	21.33 ± 1.60
Block 5 (1 plant to 12 inches)	1.01	61	236	21.33 ± 1.60
Block 6 (2 plants to 12 inches)	1.83	71	229	23.67 ± 1.65
Total (1 plant to 12 inches)	1.06	153	747	17.00 ± .81
Total (2 plants to 12 inches)	1.86	204	696	22.67 ± .94
Test 3				
Block 1 (1 plant to 12 inches)	.99	44	256	14.67 ± 1.38
Block 2 (unthinned)	5.52	161	139	53.67 ± 1.94
Block 3 (1 plant to 12 inches)	1.01	56	244	18.67 ± 1.52
Block 4 (unthinned)	5.20	92	208	30.67 ± 1.80
Block 5 (1 plant to 12 inches)	1.01	61	236	21.33 ± 1.60
Block 6 (unthinned)	6.09	99	201	33.00 ± 1.83
Total (1 plant to 12 inches)	1.01	164	736	18.22 ± .87
Total (unthinned)	5.60	352	548	39.11 ± 1.10

The smallest differences in the number of plants per foot occurred in the test of one and two plants to 12 inches, and correspondingly smaller differences in the 4-lock percentages were found. These differences, however, were greater than they had been in 1927, and the individual percentages showed wider variations. Of the total number of bolls examined in the 1-plant spacings, 17 ± 0.84 percent were 4 lock, while in the 2-plant spacings 22.67 ± 0.94 percent were 4 lock, the difference being 5.67 ± 1.26 percent, which was probably significant.

A fairly uniform response of the 4-lock percentage to the number of plants per foot was noted in the blocked-out test. The average number of plants per foot was 1.04 for the 1-plant spacings and 3.31 for the blocked out, and the percentages of 4-lock bolls were 16.33 ± 0.83 in the former spacing and 30.33 ± 1.03 in the latter, the difference being 14 ± 1.32 percent. The 4-lock percentages in both spacings were lower than in 1927, although the average number of plants per foot was higher.

Two other spacing tests were included in the 1928 work, these being the comparison of the standard 12-inch spacing with 18-inch and 24-

inch spacings, respectively. Boll counts were made in these tests, as in the others, but no significant differences in the percentages of 4-lock bolls were found.

EXPERIMENTS IN 1929

Further data on the effect of plant spacing were obtained in 1929 from the spacing tests, which consisted of comparisons of the standard 12-inch spacing with blocked-out, unthinned, 18-inch, and 24-inch spacings. The two latter tests again failed, as in the preceding year, to show any significant differences in percentages of 4-lock bolls. In the blocked-out and unthinned tests, however, effects similar to those of the two previous years were observed, with slightly greater differences between the 4-lock percentages in the wide and close spacings than in either 1927 or 1928. The data are shown in table 3.

TABLE 3 *Number of 4-lock and 5-lock bolls, percentage of 4-lock bolls, and average number of plants per row foot in spacing experiments, 1929*

Test no , block no , and treatment	Average number of plants per row foot	4-lock bolls	5-lock bolls	4-lock bolls
		Number	Number	Percent
Test 1				
Block 1 (1 plant to 12 inches)	0.97	69	231	23.00±1.64
Block 2 (blocked out)	3.31	129	171	43.00±1.93
Block 3 (1 plant to 12 inches)	.97	55	245	18.33±1.51
Block 4 (blocked out)	3.54	117	183	39.00±1.90
Block 5 (1 plant to 12 inches)	.98	75	225	25.00±1.69
Block 6 (blocked out)	3.03	92	208	30.67±1.80
Total (1 plant to 12 inches)	.97	199	701	22.11±1.93
Total (blocked out)	3.30	338	562	37.55±1.09
Test 2-				
Block 1 (1 plant to 12 inches)	.91	75	225	25.00±1.69
Block 2 (unthinned)	5.61	167	133	55.67±1.93
Block 3 (1 plant to 12 inches)	.92	62	238	20.67±1.58
Block 4 (unthinned)	5.80	159	141	53.00±1.94
Block 5 (1 plant to 12 inches)	.94	59	241	19.67±1.55
Block 6 (unthinned)	6.37	135	165	45.00±1.94
Total (1 plant to 12 inches)	.93	196	704	21.77±1.93
Total (unthinned)	5.94	461	439	51.22±1.12

In the test in which the 12-inch and blocked-out spacings were compared, the plants averaged 0.97 per foot in the former spacing and 3.30 per foot in the latter, and the 4-lock percentages were 22.11 ± 0.93 and 37.55 ± 1.09, respectively, with a difference of 15.44 ± 1.43 percent. In the unthinned blocks the plants averaged 5.94 per foot, with 51.22 ± 1.12 percent of 4-lock bolls, while in the compared 12-inch blocks they averaged 0.93 per foot, with 21.77 ± 0.93 percent of 4-lock bolls, a difference of 29.45 ± 1.46 percent. In the 12-inch spacings the 4-lock percentages were slightly higher than in 1928 and not quite so high as in 1927, whereas in the closer spaced blocks they were higher than in either of the two previous years, especially in the unthinned plants. It will be seen from table 3 that again the number of plants per foot had a marked effect on the 4-lock percentages, failing in but one instance to show a positive reaction. In the unthinned plants in block 6 there were fewer 4-lock bolls than in either of the other similarly spaced blocks, the percentage being 45 ± 1.94, with differences of 10.67 ± 2.74 and 8 ± 2.74 percent between this block and blocks 2 and 4, respectively, although the average number of plants was greater than in either of the latter.

EFFECT OF IRRIGATION AND SPACING

EXPERIMENT IN 1928

In addition to the regular spacing tests in 1928, a separate experiment was carried on to furnish additional data. Since the evidence thus far had clearly indicated that the crowding of the plants in the row had a material effect upon the proportion of 4-lock bolls produced, the question arose as to what extent other factors might be involved. The amount of moisture in the soil being a known cause of accelerated or retarded growth and being to some extent controllable under irrigation, this factor was included with the spacing of the plants.

In this experiment one of the plots at the field station was laid off into four equal parts or sections, each section being wide enough for 10 rows of plants, spaced $3\frac{1}{2}$ feet apart, or sufficient for two 4-row blocks with "guard" rows on the outer sides. In order to accentuate the difference in the spacings, an 18-inch instead of a 12-inch spacing was used in comparison with unthinned plants. In each section, five rows were thinned to the 18-inch spacing and five rows were left unthinned. Two of the sections, nos. 2 and 4, were given the normal number of irrigations during the season; the requirements being judged by the general growth and behavior of the plants. The other two sections, nos. 1 and 3, were subjected to a very restricted irrigation program, the plants being made to suffer from lack of moisture during periods of high temperature during the summer. By this arrangement the plants were grown under two widely contrasted methods of spacing and each spacing subjected to two conditions of soil moisture.

Under the normal-irrigation program, sections 2 and 4 were given six irrigations, on May 11, June 14, July 11, August 1 and 15, and September 12, respectively. Sections 1 and 3 received two less irrigations than sections 2 and 4, water being applied on May 11, July 11, August 8, and September 12.

A slight difference in the size of the plants was first noted toward the middle of July, but little effect of water stress as evidenced by wilting was apparent at that time. The extremely dry condition desired in sections 1 and 3 was interfered with to some extent by the summer rains. Several light showers occurred during May, but these were insufficient to materially affect the soil moisture, although they increased the atmospheric humidity. No rain occurred during June, but on July 19 and 20, 0.88 inch of rain fell. During August there were rains of 0.29 inch on the 1st, 0.79 inch on the 17th, and 0.34 inch on the 30th, and several light showers between. These rains relieved the plants to some extent from the extremely dry conditions that would otherwise have prevailed, and mitigated the severity of the treatment of sections 1 and 3. Some early-morning wilting of the plants in these sections, however, was noted during the first part of August, with more severe wilting during the afternoons. This was in contrast with the unwilted condition of the plants in sections 2 and 4.

Table 4 shows the data covering the results of this experiment, which were obtained in the same manner as in the regular spacing tests, 300 bolls on adjacent plants in the inside rows of each block being examined.

Effects similar to those in the regular spacing tests were encountered in this experiment, but they appeared to be influenced by irrigation.

The percentage of 4-lock bolls in the unthinned plants was higher in each case than in 18-inch-spaced plants, but the differences were greater in the sections under the light irrigation treatment than they were under normal irrigation. In the combined unthinned sections under light irrigation there was approximately 46 percent of 4-lock bolls, while in the combined 18-inch sections the percentage was approximately 20, a difference of about 26 percent. In the combined sections under normal irrigation the percentages were approximately 30 for the unthinned and 20 for the 18-inch, with a difference of a little over 10 percent. The difference in 4-lock percentage in the combined sections was thus about two and one-half times as great under light irrigation as under normal irrigation. Differences between combined sections due to irrigation treatment were about 16 percent in the unthinned plants and less than 1 percent in the 18-inch-spaced plants. From this experiment it was therefore apparent that the close spacing of the plants was a more important factor in the production of higher percentages of 4-lock bolls than the reduced amount of irrigation water, and also that the reduced moisture affected the unthinned plants to a much greater extent than the wide-spaced plants.

TABLE 4 - Number of 4-lock and 5-lock bolls, percentage of 4-lock bolls, and average number of plants per row foot, with differences due to irrigation treatment and spacing in irrigation-spacing experiment, 1928

Section no. and treatment	Average number of plants per row foot	1 plant to 18 inches			Average number of plants per row foot				Difference in percentage of 4-lock bolls due to spacing
		4-lock bolls	5-lock bolls	4-lock bolls		4-lock bolls	5-lock bolls	4-lock bolls	
		Number	Number	Percent		Number	Number	Percent	
Section 1 (light irrigation)	0.68	54	246	18.00±1.50	4.05	133	167	44.33±1.93	26.33±2.44
Section 2 (normal irrigation)	69	64	246	21.33±1.59	5.40	83	217	27.67±1.72	6.34±2.31
Section 3 (light irrigation)	69	68	232	22.67±1.63	5.69	142	158	47.33±1.94	24.66±2.53
Section 4 (normal irrigation)	70	54	246	18.00±1.50	5.11	98	202	32.67±1.83	14.67±2.37
Sections 1 and 3 (light) combined	69	122	478	20.33±1.11	4.87	275	325	45.83±1.37	25.50±1.76
Sections 2 and 4 (normal) combined	70	118	482	19.67±1.09	5.26	181	419	30.17±1.26	10.50±1.66
Difference due to irrigation treatment	--	--	--	66±1.56	--	--	--	15.66±1.86	--

EXPERIMENT IN 1929

During the season of 1929 further studies on the effect of irrigation in connection with spacing were carried on. The experiment was arranged and the plantings made as in 1928. In order, however, to accentuate the stress conditions over those of the previous season, even less irrigation water was applied to sections 1 and 3. The plants treated in this manner are referred to as "stressed," in contrast to those subjected the previous year to "light irrigation."

Under the normal method of irrigation the plants in sections 2 and 4 received five irrigations, on June 11, July 9 and 30, August 20, and September 17, respectively. Sections 1 and 3 were irrigated only twice, on June 11 and on August 6. Greater severity of water stress

was induced in these two sections than during the previous season, and there was a more notable difference in the growth and behavior of the plants. A few showers occurred during May, with a precipitation of 0.76 inch of rain on the 21st. June was without rainfall. During July 1.52 inches of rain fell on the 9th and 10th, and 0.45 inch on the 19th, with an occasional light shower toward the end of the month. On August 4, 0.24 inch of rain fell; on August 10 to 12, 1.13 inches; and on August 28 and 29, 0.69 inch. There were showers on August 8, 9, and 21, but they had little effect on the plants. During September 0.08 inch of rain fell on the 5th, 0.61 inch on the 22d, and 0.13 inch on the 24th. A greater amount of rain fell during May, July, and August than in the same months in 1928, but owing to the fewer irrigations, the plants in sections 1 and 3 were subjected to more stringent conditions. For a considerable period before the irrigation of August 6, the plants in these two sections had given indications of water shortage. They were badly wilted during most of the daylight hours, and the rate of growth and flowering was much less than in the normally irrigated sections. After the irrigation of August 6 they took on a more normal appearance, which was maintained for several days, until stress was again evidenced. On August 9 the average height of the plants in both the 18-inch and unthinned spacings of these sections was approximately 24 inches. Plants in the normally irrigated sections were from 36 to 44 inches high, the unthinned plants being a little taller than the wide-spaced plants.

A different procedure for obtaining the data was followed during the 1929 season. Since it is possible that the ultimate proportions of 4-lock and 5-lock bolls may be affected by differential shedding, as shown by Beckett and Hubbard (2), the effects of growth conditions on the number of locks or carpels may be shown more definitely in the flowers than in the bolls. It is also possible to obtain a much larger array of data from the flowers than from the mature bolls, since there is considerable shedding of squares and young bolls after flowering. Beginning on July 25, therefore, when flowering had become general, and continuing until September 27, 25 flowers in each spacing of each section were inspected daily, the number of flowers with four or five carpels being determined by the stigma lobes. By this method it was not only possible to obtain more comprehensive data, but also to follow the fluctuations in the rate of production of potential 4-lock and 5-lock bolls during the season. The subsequent tables and figures pertaining to this phase of the 1929 work, therefore, refer to the number of carpels in the flowers instead of locks in the bolls, as in the tables covering the previous work.

During the season 9,605 flowers were examined, of which 5,386 occurred in the normally irrigated sections and 4,219 in the stressed sections. When the restricted conditions of growth that prevailed at certain periods and toward the end of the season made it impossible to obtain a daily count of 25 flowers, all the flowers on the inner rows of the blocks, except those on end plants, were examined. The total number of flowers under stressed conditions comprised 2,147 from section 1 and 2,072 from section 3. Sections 2 and 4, under normal irrigation, had 2,677 and 2,709 flowers, respectively. The similarity in the number of flowers in sections 1 and 3 and sections 2 and 4 indicates that the two methods of irrigation had like effects in each of the two sections in which they were applied and that they are therefore comparable.

The number of flowers with 4 carpels and 5 carpels and the percentage of flowers with 4 carpels in the various sections and blocks are shown in table 5. In order to simplify the table, the time covered by the experiment was divided into 5-day periods, and the data are shown for such periods.

On the basis of 5-day periods it was possible to make 52 comparisons between the 18-inch and the unthinned spacings in all four sections of the experiment, or 26 comparisons in each irrigation treatment. These comparisons show that in all cases under normal irrigation, and in all but one case under the stress treatment, the unthinned plants had higher percentages of flowers with 4 carpels than the 18-inch-spaced plants. Since there were 2 days in August and 5 days in September when observations were not made, only 232 out of a possible 260 comparisons were made on the basis of daily flower counts; of these, 201 showed higher percentages of fours in the unthinned plants, 20 showed higher percentages of fours in the 18-inch-spaced plants, and 11 showed equal percentages in both spacings. Of the 116 comparisons under the stress treatment, 99 showed higher percentages of flowers with 4 carpels in the unthinned plants, 10 in the 18-inch spacing, and 7 were equal. Of comparisons in the normally irrigated sections, 102 showed higher 4-carpel percentages in the unthinned plants, 10 in the 18-inch spacing, and 4 were equal.

In the day-to-day examination of the flowers, 174 comparisons of the effects of the two irrigation treatments were afforded in each of the two spacings by comparing similar spacings in the sections which adjoined; that is, section 1 with section 2, section 2 with section 3, etc. In the 18-inch spacing, 107 of the 174 daily comparisons of the percentages of flowers with 4 carpels showed higher percentages under stress conditions, 59 under normal irrigation, and 8 were equal. In the unthinned blocks, out of the 174 daily comparisons, 121 showed higher percentages under the stress treatment, 42 under normal irrigation, and 11 were equal.

The differences between the percentages of flowers with four carpels as affected by spacing were materially greater, on the whole, than as affected by the reduction of moisture in the soil.

The data are also shown in four charts (figs. 1, 2, 3, and 4), two of which compare the figures on the basis of spacing and two on the basis of irrigation treatment, arranged by 5-day periods.

From figure 1 it will be noted that from the beginning of the daily examinations the percentages of flowers having four carpels were higher in the unthinned than in the 18-inch-spaced plants, and remained so throughout almost the entire period of observation. The percentages for the two sections of unthinned plants mounted rapidly from the end of July to the middle of August, and remained between 75 and 95 until the latter part of September. The percentages for the 18-inch spacings had a more gradual rise throughout the season until late September when, like those for the unthinned plants, they dropped very sharply. The greatest diversity between spacings in the rate of 4-carpel flower production occurred from about August 14 until about September 2, during which time the average difference was nearly 46 percent. The curves representing each of the two blocks of the same spacing in the different sections remained fairly constant in relation to each other, indicating that similar conditions prevailed in the companion blocks.

TABLE 5.--Number of flowers with 4 carpels and percentage of flowers with 4 carpels in irrigation-spacing experiment, by 5-day periods, July 25 to Sept 27, 1929

5-day period	Section 1 (stress conditions)						Section 2 (normal irrigation)					
	1 plant to 18 inches			Unthinned			1 plant to 18 inches			Unthinned		
	Flowers with 4 carpels		Flowers with 4 carpels	Flowers with 4 carpels		Flowers with 4 carpels	Flowers with 4 carpels		Flowers with 4 carpels	Flowers with 4 carpels		Flowers with 4 carpels
	Number	Percent		Number	Percent		Number	Percent		Number	Percent	
July 25 to 29	17	107	13.71±2.08	23	101	18.55±2.35	18	106	14.52±2.13	35	90	28.00±2.71
July 30 to Aug 3	19	105	15.82±2.18	28	97	22.40±2.52	13	110	10.57±1.87	30	95	24.00±2.58
Aug 4 to 8	19	51	19.00±2.65	52	48	32.00±3.37	23	77	23.00±2.54	44	36	44.00±3.35
Aug 9 to 13	46	79	36.80±2.91	74	25	74.75±2.95	24	101	19.20±2.38	90	35	72.00±2.71
Aug 14 to 18	44	79	35.77±2.92	58	10	83.28±2.89	28	97	22.40±2.52	96	35	77.42±2.53
Aug 19 to 23	45	67	40.18±3.12	22	7	75.86±5.36	41	84	32.80±2.83	73	52	58.40±2.97
Aug 24 to 28	18	25	41.86±5.07	15	3	58.33±5.92	30	70	30.00±3.09	55	45	55.00±3.37
Aug 29 to Sept 2	8	5	61.54±9.09	11	1	91.67±5.38	35	90	28.00±2.71	79	41	65.83±2.92
Sept 3 to 7	51	29	63.75±3.63	50	10	53.33±3.24	34	91	27.20±2.69	80	45	64.00±2.90
Sept 8 to 12	58	41	58.59±3.34	91	9	91.00±1.93	45	43	51.14±3.59	52	26	66.67±3.60
Sept 13 to 17	66	31	68.04±3.19	94	6	94.00±1.60	52	13	80.00±3.35	70	12	85.37±2.63
Sept 18 to 22	55	9	83.33±3.42	70	5	98.33±1.94	25	13	65.79±5.19	7	7	88.89±2.67
Sept 23 to 27	22	21	51.16±5.14	48	52	48.00±3.37	17	17	90.00±5.79	64	24	72.73±3.20
Total	458	679	40.28±.98	636	374	62.97±1.02	385	912	29.68±.86	824	556	59.71±.89

TABLE 5.—Number of flowers with 4 carpels and 5 carpels and percentage of flowers with 4 carpels in irrigation-spacing experiment, by 5-day periods, July 25 to Sept. 27, 1929—Continued

5-day period	Section 3 (stress conditions ¹)				Section 4 (normal irrigation)			
	1 plant to 15 inches		Unthinned		1 plant to 18 inches		Unthinned	
	Flowers with—		Flowers with—		Flowers with—		Flowers with—	
	4 carpels	5 carpels	4 carpels	5 carpels	4 carpels	5 carpels	4 carpels	5 carpels
July 25 to 29.....	Number	Percent	Number	Percent	Number	Percent	Number	Percent
July 30 to Aug 3.....	13	112	10	40±1.84	28	96	28	96
Aug 4 to 8.....	12	113	9	60±1.78	36	88	31	94
Aug 9 to 13.....	23	17	23	100±2.84	44	56	44	83
Aug 14 to 18.....	24	101	19	20±2.58	39	13	17	83
Aug 19 to 23.....	40	67	23	20±2.55	23	2	76	49
Aug 24 to 28.....	12	27	37	58±3.16	14	1	114	11
Aug 29 to Sept 2.....	12	12	36	88±5.65	19	106	86	39
Sept 3 to 7.....	32	12	58	62±6.16	24	76	46	54
Sept 8 to 12.....	30	30	43	62±6.16	31	94	60	65
Sept 13 to 17.....	70	27	43	60±3.94	33	33	61	65
Sept 18 to 22.....	51	27	72	16±3.07	54	32	72	21
Sept 23 to 27.....	31	8	82	16±3.01	27	17	70	8
Total.....	404	751	34	98±	570	347	83	585

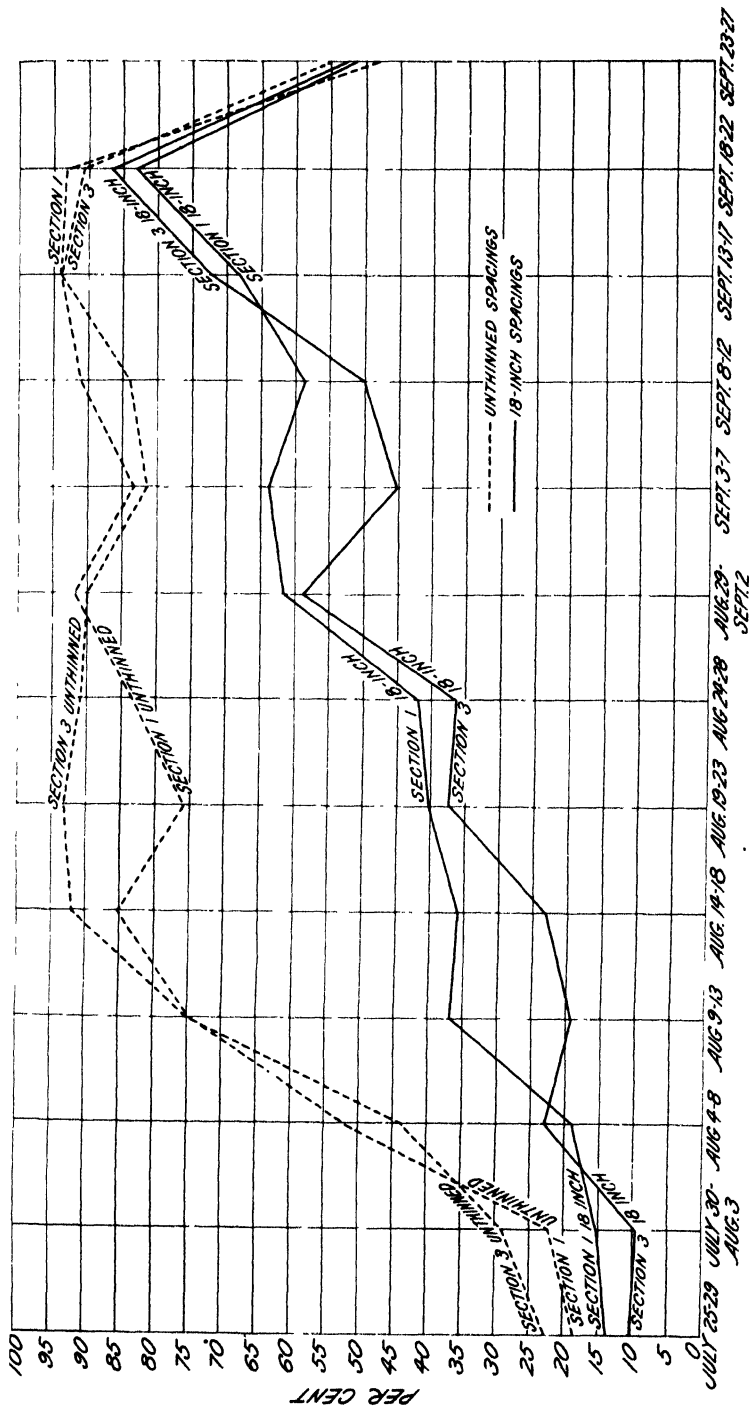


FIGURE 1.—Percentages of flowers with 4 carpels in 18-inch and unthinned spacings, sections 1 and 3 stress treatment, by 5-day periods, in irrigation-spacing experiment July 25 to Sept 27, 1929.

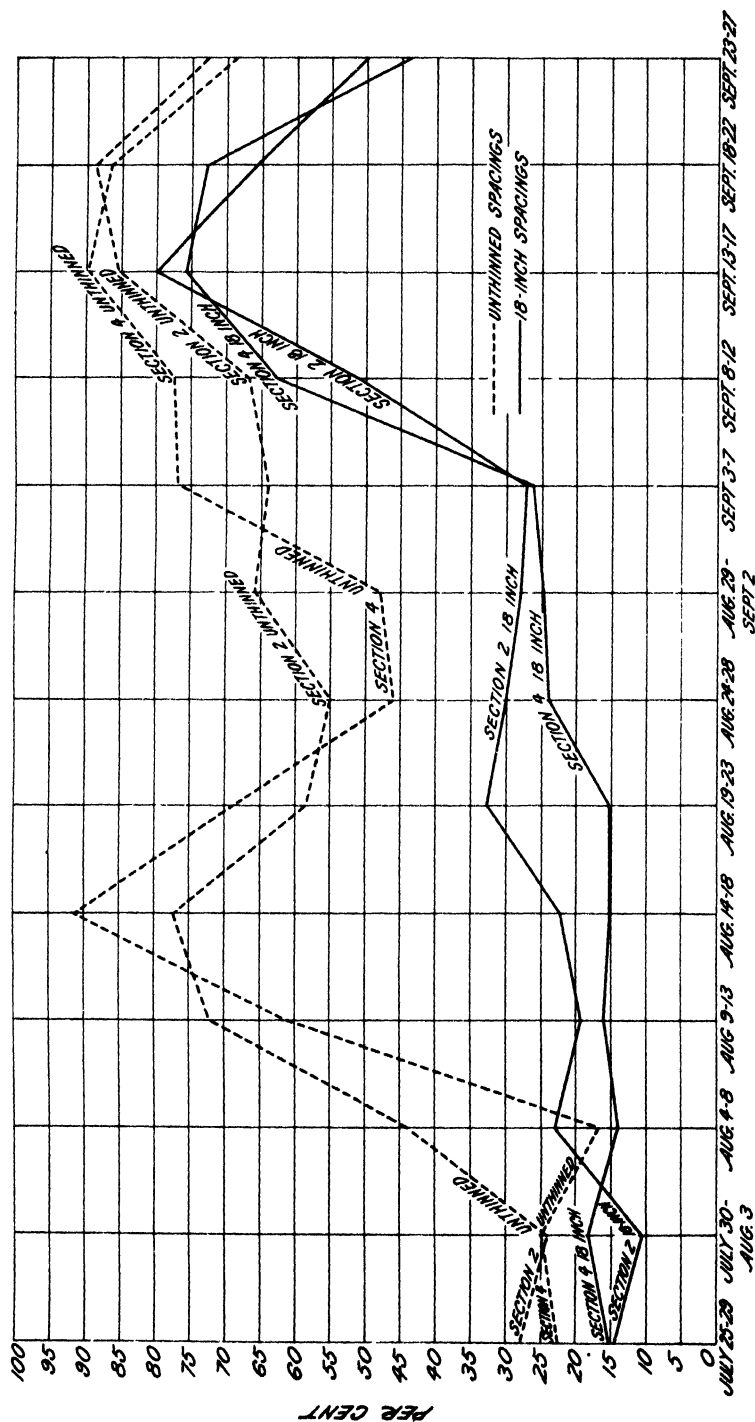


FIGURE 2.—Percentages of flowers with 4 carpels in 18-inch and unthinned spacings, sections 2 and 4, normal irrigation, by 5-day periods, in irrigation-spacing experiment, July 25 to Sept 27, 1924.

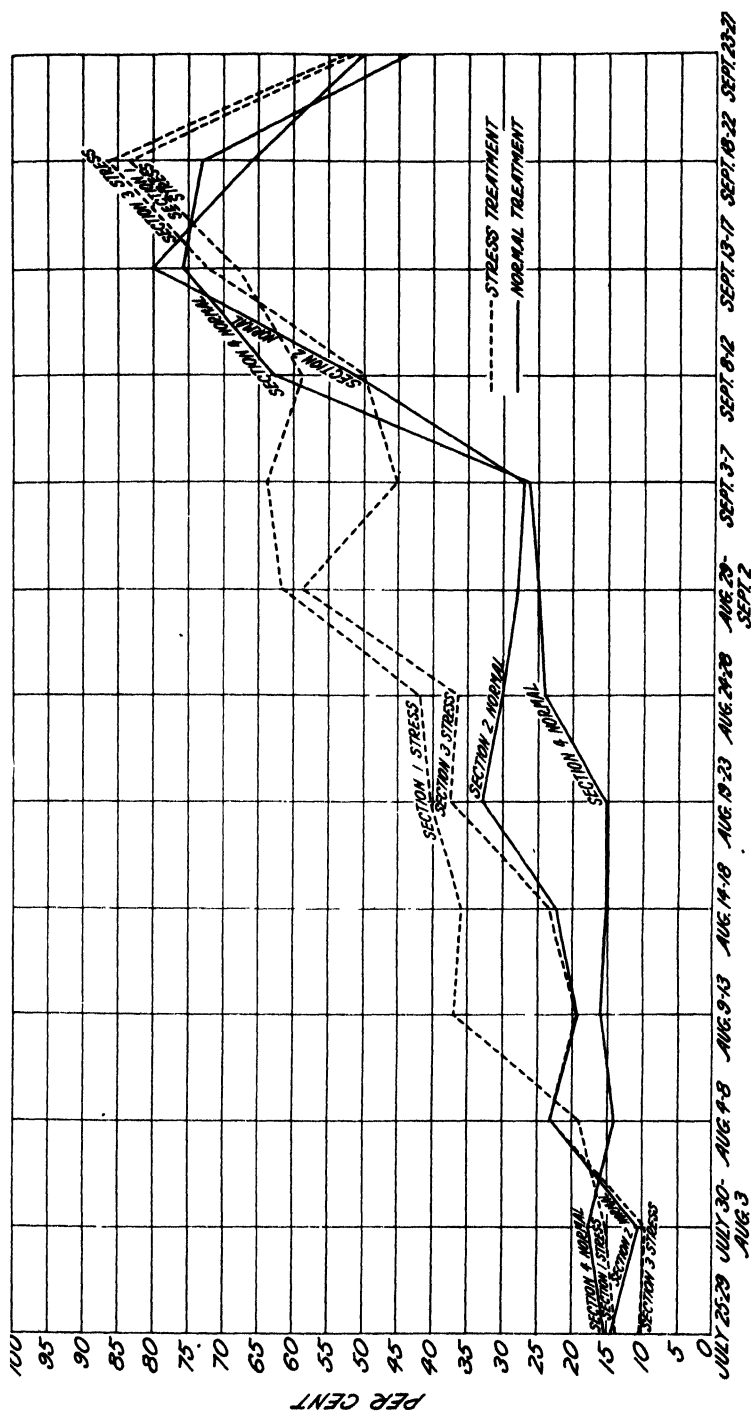


FIGURE 3.—Percentages of flowers with 4 carpels under normal irrigation and stress treatment in 18-inch spaced plants, sections 1, 2, 3, and 4, by 5-day periods, in irrigation spacing experiment, July 25 to Sept. 27, 1929.

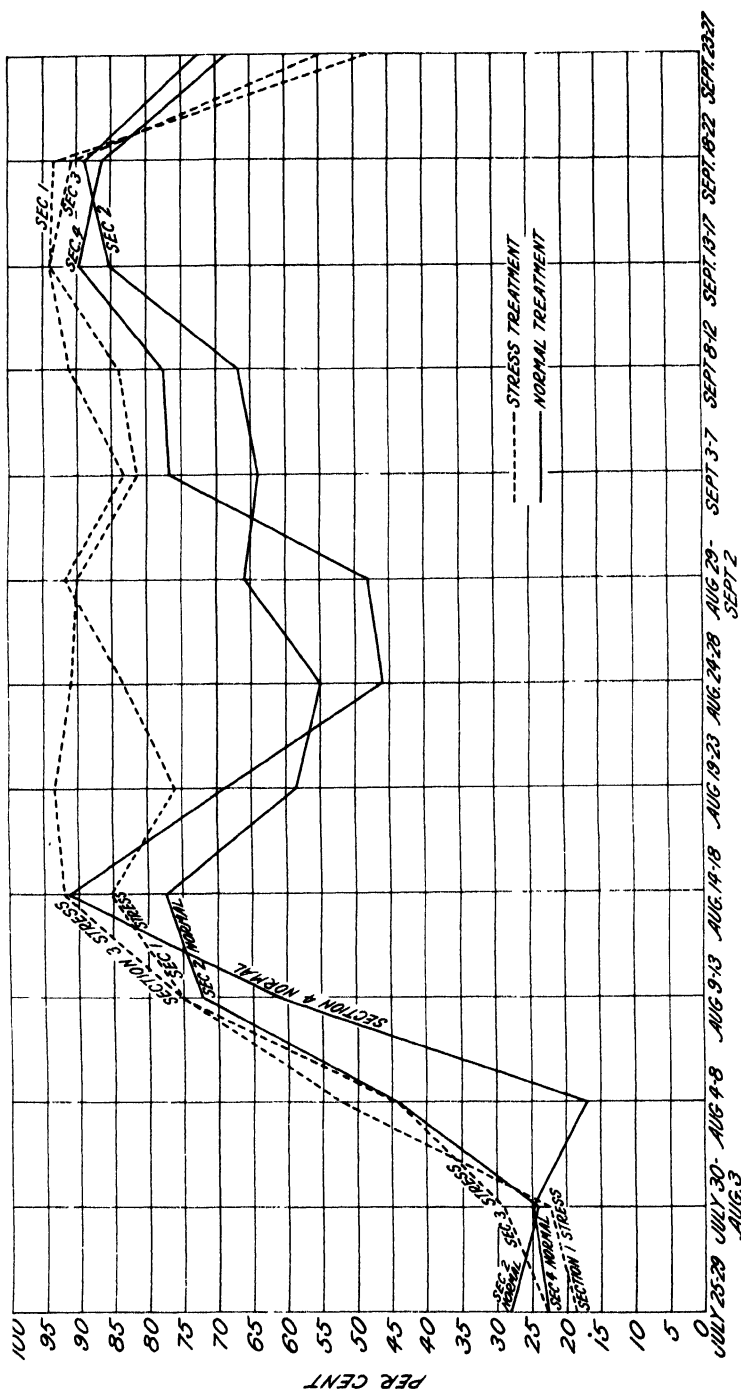


FIGURE 4.—Percentages of flowers with 4 carpels under normal irrigation and stress treatment in unthinned plants; sections 1, 2, 3, and 4, by 5-day periods, in irrigation-
treatment experiment. Table of the same for 1938.

During the entire season the percentages of flowers with four carpels were not so high for either spacing under normal irrigation (fig. 2) as under stress conditions (fig. 1). The percentage of fours in the 18-inch spacings remained fairly low from July 25 to about September 7, when it averaged approximately 27. During the ensuing 10-day period there were sharp rises in the percentages in both sections until the average was approximately 78, a difference of over 50 percent. From this peak of 4-carpel flower production, about September 17, the percentage dropped to approximately 47 on September 27, a difference of over 30 percent. In the unthinned plants the 4-carpel percentages rose very rapidly in the early part of the season, increasing from approximately 24 percent in the period from July 30 to August 3 to an average of over 84 percent by August 18, a difference of over 60 percent. The percentages then declined sharply during the last two 5-day periods. The reasons for sudden rises and falls in the 4-carpel percentages are undetermined, but are probably associated with cultural conditions previously encountered by the plants.

In figure 3 the data have been arranged to show the effect of the contrasting moisture conditions upon the production of flowers with four carpels in the 18-inch-spaced plants. The curves indicate that during the 15 days after observations were begun there were only slight differences in the percentages in the two treatments. After August 8, however, the stressed plants began to produce more flowers with four carpels, and this was continued for over a month. The marked rises in the percentages in the stressed sections after August 28 were not followed by a rise in the percentages in the normally irrigated sections, where for 10 days longer the percentages remained low. A very sharp rise then occurred in the percentages in the normally irrigated blocks, until for the only time during the season they exceeded those in both the stressed sections. This condition lasted for but a few days, when the percentages under the normal treatment again fell below those under the stress treatment.

Figure 4 shows the percentages of 4-carpel flowers in the unthinned plants under the two irrigation treatments in the same manner that figure 3 shows those in the 18-inch spacings. By comparing figures 3 and 4, it will be seen that the percentages of flowers with 4 carpels were higher in the unthinned plants throughout most of the season. As between irrigation treatments (fig. 4), the percentages in the stressed sections were somewhat higher than in the normally irrigated sections, with very pronounced differences occurring during the middle and latter part of August and early September. Toward the end of the season the percentages became fairly uniform for about 10 days and then dropped sharply, when the stressed sections showed greater reductions than did the normally irrigated sections.

During the earlier part of the season the percentages of flowers with 4 carpels in both the normally irrigated and stressed sections increased much more rapidly in the unthinned plants than in the 18-inch spacings. During the middle of August, the proportion of fours in the unthinned plants was very high, particularly in the stressed sections. The 18-inch spacings, on the whole, showed a more gradual rise in 4-carpel percentages as the season progressed, which may be considered a normal occurrence. From September 23 to 27 a marked drop occurred in the percentages in both spacings and both irrigation treatments. This sharp reduction in the proportion of 4-carpel

flowers in all the plants, regardless of spacing or irrigation treatment, apparently resulted from the same cause, which was probably physiological.

A comparison of figures 1 and 2 with figures 3 and 4 shows that during most of the season the proportion of 4-carpel flowers produced depended upon spacing to a considerably greater extent than upon irrigation. In other words, in the production of flowers having four carpels, the crowding of the plants was a more important factor than the stress conditions resulting from reduced soil moisture.

Table 6 shows the average number of plants per foot and the average percentages of flowers with four carpels under the different spacing and irrigation treatments for the entire season of 1929. The differences in the percentages due to irrigation were relatively small, only 3.59 ± 0.97 in the unthinned plants and 9.31 ± 0.91 in the 18-inch spaced plants, the stressed sections having the higher percentages in both cases. However, it is probable that the small percentage difference in the unthinned plants was partly due to the fact that the normally irrigated sections were somewhat more crowded, as they averaged one plant more to the foot than the stressed sections. The effect of stress conditions appears to have been greater in the wide-spaced than in the crowded plants, since the difference in the 4-carpel percentage was nearly three times as large in the former as in the latter, but it is likely that this difference would have been less had the unthinned plants under stress treatment been as closely spaced as those under normal irrigation. The differences resulting from irrigation treatment were much smaller than those due to plant spacing. There was a difference of 24.97 ± 1.00 between the 18-inch and unthinned plants in the percentage of flowers with 4 carpels under the stress treatment, and under normal irrigation the difference was 30.69 ± 0.87 , the unthinned plants having the higher percentages.

TABLE 6.—Percentage of flowers with 4 carpels and average number of plants per row foot, with differences due to irrigation treatment and spacing, covering the entire season, in irrigation-spacing experiment, 1929

Section no. and treatment	18-inch spacing		Unthinned		Difference due to spacing
	Average number of plants per row foot	Percentage of flowers with 4 carpels	Average number of plants per row foot	Percentage of flowers with 4 carpels	
Sections 1 and 3 (stress) combined.....	0.66	37.61 $\pm$ 0.68	4.78	62.58 $\pm$ 0.74	24.97 $\pm$ 1.00
Sections 2 and 4 (normal) combined.....	.67	28.30 $\pm$.60	5.78	58.90 $\pm$.63	30.69 $\pm$.87
Difference due to irrigation treatment....		9.31 $\pm$.91		3.59 $\pm$.97	

A comparison of table 6 and table 4 shows that the percentages of flowers with 4 carpels in 1929 was greater than the 4-lock boll percentages in 1928, although the averages of the number of plants per foot were but slightly different in the two years. The difference in the percentages was most apparent in the normally irrigated sections, especially among the unthinned plants. As already mentioned, similar differences were encountered in the regular spacing tests for these two years. It will be noted that the effects of the irrigation treatments apparently differed widely in the two seasons. In 1929 the difference in the percentages of flowers with 4 carpels, due to the method of irrigation, was much greater in the 18-inch-spaced than in

the unthinned plants; whereas in 1928 there was practically no difference due to irrigation treatment in the 18-inch-spaced plants, and the unthinned plants showed a difference of about 15 percent. This apparent reversal can not be explained, but it was probably due in part to differences in seasonal conditions. Moreover, the data for the two seasons were secured by different methods, one table being based on 300 bolls without regard to the date of their appearance and the other on a considerably larger number of flowers, which appeared during the growing season.

SUMMARY

Practically all the bolls produced by American upland varieties of cotton have either 4 or 5 locks or carpels. The number of locks, although regarded as a heritable character, is affected by cultural conditions. Several investigators have noted the effects of changed or unfavorable conditions of growth upon various characters of the cotton plant. Among the effects observed was reduction in the number of locks per boll.

An example of the effect of close spacing or crowding of cotton plants upon the number of locks was observed by the writers in 1926, in experiments at the United States Acclimatization Field Station at State College, N.Mex. In two of these experiments it was found that the closer spacings consistently produced higher proportions of 4-lock bolls, and these relations were studied more intensively in the following year. Comparisons of lock numbers were made in four spacings: Single plants 12 inches apart in the row; two plants to 12 inches; plants left in groups, or "blocked-out"; and plants left without thinning. No difference was shown between one and two plants at 12 inches, but the experiment with blocked-out plants showed 10 percent more 4-lock bolls than the 12-inch spacing, while the plants that were not thinned showed nearly 17 percent more 4-lock bolls. Numerous blocks were compared and as a general rule the percentage of 4-lock bolls was higher or lower as the number of plants per foot in the rows increased or diminished. Further data along the same lines were obtained in 1928.

In addition to observations in the regular spacing tests in 1928, another experiment was conducted for studying the combined effects of different irrigation and spacing treatments in relation to the production of 4-lock bolls. Two widely different spacings of the plants and two methods of irrigation were employed in this experiment, the spacings being (1) one plant to 18 inches and (2) unthinned plants, and the irrigation methods being (1) normal and (2) "light." In the light-irrigation method, the moisture supplied to the plants was less than is normally required. It was again found that the closer spaced plants had produced considerably higher percentages of 4-lock bolls, but it was also evident that the irrigation practice had had an effect, the light-irrigated plants producing more 4-lock bolls than those which received a normal amount of water. The effect of spacing, however, was evidently greater than that of irrigation.

In 1929 the effect of spacing in the regular tests was again studied, and similar data were obtained as in the two preceding years. In addition, the combined spacing and irrigation experiment was repeated, and the difference in irrigation treatments was intensified by applying still less water to some of the plants. Since it is possible

that on account of differential shedding of 4-lock and 5-lock bolls the effects of cultural conditions may be shown more definitely in the flowers than in the bolls, and in order to obtain a larger array of data, a different procedure was followed. Instead of waiting until the end of the season a daily examination was made of the flowers as they bloomed, the number of carpels being determined by the stigma lobes. On the basis of daily flower counts a large number of comparisons between spacing treatments was made and in much the greater number of cases the close-spaced plants produced more flowers with 4 carpels. Similar daily comparisons of the effects of irrigation treatment showed higher percentages of 4-carpel flowers among the plants under restricted moisture conditions, although in not so many instances as under the different spacings. Average percentages of flowers with four carpels covering the entire season as affected by spacing showed differences of nearly 25 percent in the stressed sections and a little more than 30 percent in the normally irrigated sections, while under the different irrigation treatments the differences were about 3½ percent in the unthinned plants and a little over 9 percent in the 18-inch spaced plants.

The data, on the whole, indicate that the amount of space available for the development of the plants has a material influence upon the proportion of 4-lock bolls produced; crowding the plants in the rows reduces the percentage of 5-lock bolls. The amount of moisture in the soil is also a determining factor, although apparently it is of less importance than the spacing of the plants.

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LYSIMETER EXPERIMENTS WITH SULPHATE OF AMMONIA AND NITRATE OF SODA¹

By T. L. LYON, *professor of soil technology and head of Department of Agronomy*,
and J. A. BIZZELL, *professor of soil technology, New York (Cornell) Agricultural
Experiment Station*

INTRODUCTION

An advantage sometimes attributed to sulphate of ammonia as a nitrogenous fertilizer when compared with nitrate of soda is that it does not occasion so large a loss of nitrogen in drainage water as does the nitrate. It was decided to submit this to trial in the lysimeter tanks at this experiment station. As the drainage from these tanks consists of the percolation of natural rainfall, a test covering a period of 10 or more years should demonstrate what could be expected from a given soil under a rainfall of 30 to 35 inches.

PREVIOUS INVESTIGATIONS

There have been a number of investigations of this subject, but few of them have extended over any considerable period of years. Some of the experiments have been conducted with soils on which plants were grown; others have been carried on with moist and bare soils which were allowed to stand for longer or shorter periods in a percolation apparatus of some kind and then leached. The literature bearing on this subject was reviewed by Bizzell² in 1926. Since then some further work has been reported. In general the results indicate that more nitrogen is found in the drainage water from soil treated with nitrate of soda than from similar soil which received an equivalent quantity of nitrogen in the form of sulphate of ammonia. At least such findings are reported by Collison and Walker (4), Hall (7, p. 235-236), Morgan, Street, and Jacobson (9), Collison (3, p. 815), Geilmann (6), Eckart (5), and Tidmore and Williamson (12, p. 38-41). The opposite result was obtained by Peck (10) and by Geilmann (5) when working with clay soils. Geilmann attributes this result to the deflocculating action of the large quantity of nitrate of soda used in the experiment which prevented the soil so treated from draining properly, and in consequence the volume of drainage was small as compared with that of the soil treated with sulphate of ammonia.

Experiments dealing with the effect of these two fertilizers on the removal of calcium in the drainage water have been reported by Hall and Miller (8), Morse (10), Morgan, Street, and Jacobson (9), Eckart (5), and Peck (11). All of these investigators found more calcium in the drainage water from the soil to which sulphate of ammonia was applied than from soil treated with nitrate of soda, when both were used in amounts containing equivalent quantities of nitrogen.

¹ Received for publication Jan. 26, 1933, issued August 1933.

² Reference is made by number (italic) to Literature Cited, p. 63

EXPERIMENTAL PROCEDURE

Six tanks, each having a capacity of $3\frac{1}{2}$ tons of soil, a depth of 4 feet, and an area somewhat more than 4 by 4 feet, were used for the experiment. The tanks were filled with Dunkirk sandy gravelly loam, the surface soil and subsoil being placed in the order of their occurrence in the field. As its name connotes this soil is a sandy one, only weakly buffered, well aerated, and readily permeable to water. It is a type of soil that is frequently used for market gardening as it responds readily to commercial fertilizers. The tanks were filled with soil in 1921, and the following year the cropping system was begun.

At the beginning of the experiment three nitrogen carriers were used. These included dried blood in addition to sulphate of ammonia and nitrate of soda. After 1925 the use of dried blood was discontinued. Of the two tanks to which it had been applied (nos. 1 and 22), one afterwards received sulphate of ammonia and the other nitrate of soda. It was thought that by giving each a different treatment they could be continued in the experiment without favoring one or the other of the two nitrogen carriers remaining in the test. Moreover, by retaining these tanks in the experiment each treatment could be made in triplicate.

In order to insure a removal of nitrogen in measurable quantities, the applications of nitrogenous fertilizers were large, and to avoid injury to the growing plants the applications were made twice during the season. For this reason it was desirable to have two crops each year, and this could best be accomplished by using market-garden crops. One application was accordingly made in the spring and the other before the second crop was planted in July.

At the outset of the experiment the fertilizers applied each year contained 82.35 pounds of nitrogen per acre. Later the nitrogen was increased to 164.7 pounds per acre each year. This increase in nitrogen was found to be necessary in order to insure an excess over the quantities used by the crops. Somewhat later the quantity of muriate of potash was also increased to insure an excess of potassium, and the quantity of superphosphate was slightly reduced. Whereas the muriate of potash was applied at the rate of 100 pounds per acre at the beginning it was increased to 200 pounds, and the superphosphate, which was used at the rate of 500 pounds, was later decreased to 400 pounds for each crop.

The soil in the tanks was an acid one, and it was considered best to lime it to make it more favorable for nitrification. Accordingly, ground limestone was applied to all tanks at the rate of 4,000 pounds to the acre before the first crop was planted in 1922. The limestone contained 95 percent calcium carbonate and 2 percent magnesium carbonate and was so ground that 94 percent would pass through a $\frac{1}{16}$ -inch opening. In the spring of 1926 all the tanks were again limed, this time at the rate of 2,000 pounds to the acre. From that time on only the tanks which received sulphate of ammonia were limed. It was not until 1927 that the tanks treated with ammonium sulphate began to increase in acidity. It then became difficult to prevent the soil in these tanks from becoming strongly acid. In the spring of 1928 another application 2,000 pounds per acre of ground limestone was given to tanks 1, 21, and 24, and in the fall 4,000 pounds

per acre was applied. Again in 1930 an application of 4,000 pounds per acre of ground limestone was made. The total quantity of ground limestone used on the soil that received nitrate of soda was therefore at the rate of 3 tons per acre and on the ammonium sulphate-treated soil 8 tons.

DETERMINATION OF THE SOIL REACTION

Determinations of the hydrogen-ion concentration were made from time to time, this being done more frequently for the sulphate of ammonia soil. The results are shown in table 1.

TABLE 1.—*Hydrogen-ion concentration in soil on different dates, in tanks receiving sulphate of ammonia or nitrate of soda*¹

Tank no.	Nitrogen carrier	Apr 21, 1926	Apr 1, 1927	Nov 23, 1927	July 12, 1928	Oct 31, 1928	Apr. 16, 1930	Nov 25, 1930	May 2, 1931	Nov 2, 1931
1	Sulphate of ammonia	pH 6.4	pH 7.4	pH 6.6	pH 6.5	pH 6.1	pH 7.3	pH 7.2	pH 7.0	pH 7.0
21	do.	6.3	6.6	6.0	6.1	5.8	7.2	7.2	6.9	6.9
24	do.	6.3	7.3	5.7	6.2	5.8	6.6	6.9	6.8	6.8
2	Nitrate of soda	6.5	7.9	7.8			8.1		7.7	7.5
22	do.	6.4	7.8	7.7			7.8		7.6	7.6
23	do.	6.5	7.8	7.7			7.9		7.7	7.6

¹ In the spring of 1928 ground limestone was applied to tanks 1, 21, and 24 at the rate of 2,000 pounds per acre, and the following fall an application was made at the rate of 4,000 pounds per acre. In 1930 another application was made at the rate of 4,000 pounds per acre.

The determinations of April 21, 1926, were made by the colorimetric method, all the others were made with a potentiometer. Samples were taken from the surface 6 inches of soil. From table 1 it will be seen that there was a strong tendency for the soil that received sulphate of ammonia to become acid. Most of the time the hydrogen-ion concentration in this soil was maintained at a pH of 6.0 or above, but it was only by applying enormous quantities of ground limestone that it could be kept near neutrality. As will be shown later, the quantity of nitrogen applied was not greatly in excess of that removed by the crops and drainage. It was, therefore, not much more than good management would require.

THE CROPPING SYSTEM

Four kinds of plants were used for cropping the soil. Two of these were grown one year and the other two the next year. Thus in 1922 spinach was planted in the spring and carrots in the summer. After the carrots were harvested rye was planted and remained on the land until turned under the following spring in preparation for the next planting. In 1923 lettuce was planted in the spring and this was followed by garden beets. Rye followed the beets and was turned under in the spring. This system was continued until May 1, 1931, at which time the drainage water was collected for the last period recorded in this report.

The crops grew normally. In time of drought enough rain water from the cistern was applied to prevent loss of plants and to maintain growth. All tanks received the same amount of rain water. When seed for the second crop was planted, the ground was usually shaded with muslin to diminish evaporation and protect the young plants. In this way a good stand was obtained.

The drainage water for any year consisted of the quantity collected between May 1 of one year and May 1 of the next. This has been done in all the lysimeter experiments at this station because the drainage water collected during the early spring is greatly affected by the soil treatment of the previous summer. There is little percolation in this climate during the fall and winter months but much when the ground thaws in March and April.

Two constituents only were determined in the drainage water. These were nitrates and calcium. For the former the phenol-disulphonic acid method was used, and for the latter the official method of the Association of Official Agricultural Chemists (1). Practically all of the nitrogen in the drainage water was in the form of nitrates.

Drainage water was measured in the receiving cans, each of which had a side tube calibrated to show the contents in liters. An aliquot sample was removed whenever there was enough water to warrant it, and the sample was placed in an acid bottle kept slightly acid with H_2SO_4 to prevent settling out of calcium carbonate.

In the crops dry matter, total nitrogen, and calcium were determined. The official methods of the Association of Official Agricultural Chemists (1) were used for these determinations.

EXPERIMENTAL RESULTS

DRY MATTER AND NITROGEN IN THE CROPS

The effect of the nitrogenous fertilizers on the yield of dry matter and nitrogen in the 20 crops is shown in table 2.

TABLE 2.—*Dry matter and nitrogen in the 20 crops grown in soil treated with sulphate of ammonia and nitrate of soda, 1922 to 1931*

Tank no	Nitrogen carrier	Dry matter			Nitrogen	
		Pounds per acre	Pounds per acre	Percent		
1	Sulphate of ammonia	44,359	937	2.11		
21	do	45,232	962	2.13		
24	do	45,352	981	2.16		
2	Nitrate of soda	53,507	1,069	2.00		
22	do	52,513	1,048	1.99		
23	do	55,728	1,146	2.06		

It is quite evident from table 2 that the crop yields were materially larger in the tanks treated with nitrate of soda and that the quantity of nitrogen removed from the soil was almost correspondingly large. The percentage of nitrogen in the dry matter was slightly higher in the crops grown with sulphate of ammonia. In view of the relative quantities of nitrogen removed in drainage water from the tanks treated with the two nitrogen carriers, which will be shown later, this difference in the amount of nitrogen removed in the crops is interesting.

RELATION OF CROP YIELDS TO HYDROGEN-ION CONCENTRATION IN THE SOIL

While the acidity was greater in the soil treated with sulphate of ammonia than in the soil treated with nitrate of soda during a part of the time consumed by the experiment, such was not always the

case. During the first 4 years there was practically no difference in the soil reaction, as is indicated by the pH determinations made on April 21, 1926. The soil in all tanks had the same reaction at the outset and must have maintained that relation up to the spring of 1926. From 1922 to 1925, inclusive, tanks 21 and 24 received sulphate of ammonia and tanks 2 and 23 received nitrate of soda. The yields of dry matter in the eight crops grown in these tanks during this period are given in table 3.

TABLE 3.—Average annual yield of dry matter from crops in tanks receiving sulphate of ammonia or nitrate of soda, 1922-25 and 1930-31

[Data expressed as pounds per acre]

1922-25			
Tank no		Yield from tanks receiving sulphate of ammonia	Yield from tanks receiving nitrate of soda
21		4,325	2
24		4,203	23
Average		4,264	5,118
Ratio		100	117
1930-31			
21 ^a		5,240	^b 2
24 ^a		5,173	^b 23
Average		5,206	6,174
Ratio		100	119

^a pH from 6.8 to 7.3

^b pH from 7.6 to 8.1

The data in table 3 show that the larger yields were in the tanks which received nitrate of soda. The larger yields in tanks 2 and 23 could not be said to be due to differences in the hydrogen-ion concentration of the soil.

Several investigators have found that a marked acidity in soil curtails the effectiveness of sulphate of ammonia, and it was therefore thought that a degree of acidity indicated by a pH value of 6.3 to 6.5 might be sufficient to produce an injurious effect on the growth of the plants in tanks 21 and 24. Accordingly, in 1930 and 1931 the reaction of the soil in these tanks was brought to a point represented by a pH value of 6.8 to 7.3, while that in tanks 2 and 23 was 7.6 to 8.1. As the soil in tanks 2 and 24 had now approached neutrality the reaction should not interfere with the availability of the nitrogen in sulphate of ammonia. The yields of dry matter in the crops grown in tanks 21, 24, 2, and 23 are given in table 3.

The yields of dry matter recorded in table 3 do not indicate that the sulphate of ammonia as compared with nitrate of soda was any more effective when used on a soil with a pH value of 6.8 to 7.3 than when used on a soil with a pH value of 6.3 to 6.5.

NITROGEN IN THE DRAINAGE WATER

All the drainage water that percolated through the soil of these tanks during 9 years was collected, measured, and analyzed for nitrogen and calcium. Nitrogen appeared almost entirely in the form of nitrates. The total quantity of nitrogen from each tank is shown in table 4.

TABLE 4. - *Nitrogen in drainage water from tanks receiving sulphate of ammonia or nitrate of soda, 1922-31*

[Data expressed as pounds per acre]

Tank no.	Nitrogen carrier	Total nitrates	Nitrogen	
			Total	Average annual
1.....	Sulphate of ammonia.....	1,236.00	279.34	31.04
21.....	do.....	1,204.82	272.29	30.25
24.....	do.....	1,034.58	233.82	25.98
Average.....				29.09
2.....	Nitrate of soda.....	1,600.71	361.76	40.19
22.....	do.....	1,620.01	366.12	40.68
23.....	do.....	1,419.31	320.76	35.64
Average.....				38.84

The large quantity of nitrogenous fertilizers applied and the sandy nature of the soil combined to cause a large removal of nitrogen in the drainage water. The figures obtained were several times greater than those for nitrogen previously found by the authors in the drainage from a silty clay-loam soil that had received no fertilizer other than a moderate quantity of farm manure.

It is apparent from table 4 that the loss of nitrogen in the drainage was more than 30 percent greater from the tanks treated with nitrate of soda than from those treated with sulphate of ammonia.

It will be recalled that less nitrogen was removed by crops from the soil treated with sulphate of ammonia than from that treated with nitrate of soda. It appears, therefore, that both crops and drainage water from soil receiving nitrate of soda carry off more nitrogen than they do from soil receiving an equivalent quantity of nitrogen in the form of sulphate of ammonia. This is brought out in table 5.

TABLE 5.—*Average quantity of nitrogen removed annually in crops and drainage water from tanks receiving sulphate of ammonia or nitrate of soda, 1922-31*

[Data expressed as pounds per acre]

Tank nos.	Nitrogen carrier	Nitrogen applied—			Nitrogen removed—		
		In fertilizer	In rain-fall	Total	In crops	In drainage	Total
1, 21, 24.....	Sulphate of ammonia.....	130	6.5	136.5	96.0	29.1	125.1
2, 22, 23.....	Nitrate of soda.....	130	6.5	136.5	108.7	38.8	147.5

Table 5 shows that the nitrogen removed in crops and drainage combined was considerably less for the soil treated with sulphate of ammonia than for that treated with nitrate of soda. The quantity of nitrogen applied in fertilizers was somewhat more than the total amount removed from the sulphate of ammonia tanks and less than that removed from the nitrate of soda tanks. In addition to the nitrogen supplied in the fertilizers, the amount brought down by the atmospheric precipitation amounted to 6.5 pounds to the acre.

NITROGEN IN CROPS AND NITRATES IN DRAINAGE WATER IN SUCCESSIVE YEARS

As was to be expected, there were large seasonal variations in crop yields and also in seasonal removal of nitrates from the soil. That there was a fairly uniform relation between these two phenomena is brought out by table 6. Data from tanks 21, 24, 2, and 23 are given because these tanks were treated with the same nitrogen carrier throughout the experiment.

TABLE 6 — *Nitrogen in crops and nitrates in drainage water, during successive years from tanks receiving sulphate of ammonia or nitrate of soda*

[Data expressed as pounds per acre]

Year	Nitrogen in crops		Nitrates in drainage	
	Tanks receiving sulphate of ammonia (nos. 21, 24)	Tanks receiving nitrate of soda (nos. 2, 23)	Tanks receiving sulphate of ammonia (nos. 21, 24)	Tanks receiving nitrate of soda (nos. 2, 23)
1922	52	51	173	220
1923	107	110	33	34
1924	100	118	38	50
1925	90	111	37	43
1926	67	81	300	367
1927	122	159	104	76
1928	76	88	221	319
1929	149	142	44	54
1930	71	92	169	348

The most evident relationship to be seen is that in the years when the crops contained a large number of pounds of nitrogen the quantities in the drainage water were small, and, conversely, when the crops contained little nitrogen the drainage water contained a great deal. This is what might be expected when weather conditions curtail plant growth. Under such circumstances the nitrate nitrogen not absorbed by the plants is carried off in the drainage water.

In every year except one there was more nitrate in the drainage from the nitrate of soda tanks than from those that received sulphate of ammonia. In years when there were small amounts of nitrogen in the crops the removal of nitrogen in the drainage water was such as to entail a considerable monetary loss. This was especially true when the soil was treated with nitrate of soda.

The largest removal of nitrogen in drainage in any one year amounted to 68 pounds to the acre from the sulphate of ammonia tanks and 83 pounds to the acre from the nitrate of soda tanks.

CALCIUM IN CROPS AND IN DRAINAGE WATER

Determinations of calcium were made in the crops and in the drainage water in order to ascertain the relative effects of the two carriers of nitrogen on the removal of this constituent from the soil. Table 7 shows the average amount of calcium removed annually from each tank during the period 1922 to 1931, inclusive. The records for 20 crops and the drainage records for 9 years are included in the table.

TABLE 7.—Average quantity of calcium removed annually in crops and drainage water from tanks receiving sulphate of ammonia or nitrate of soda, 1922-31

[Data expressed as pounds per acre]

Tank no	Nitrogen carrier	Calcium removed	
		In crops	In drainage water
1	Sulphate of ammonia	40.8	357.7
21	do	41.7	385.3
24	do	44.6	359.0
	Average	42.4	367.3
2	Nitrate of soda	31.5	279.2
22	do	34.5	290.7
23	do	34.5	265.8
	Average	33.5	278.6

It is apparent that the crops produced on the soil which was treated with sulphate of ammonia contained more calcium than did those on the soil treated with nitrate of soda, and this despite the fact that larger crops were produced on the latter soil. The drainage water from the ammonium sulphate soil also contained more calcium than that from the nitrate of soda soil. It seems rather curious that with such excessive quantities of soluble calcium in these tanks, as is indicated by the figures for calcium in drainage water, there should have been any greater absorption of calcium by the plants in the sulphate tanks than in the others. It will be remembered, however, that more lime was applied to the sulphate of ammonia tanks than to those that received nitrate of soda. This may account for the larger quantity of calcium in the plants and in the drainage water of the tanks that received sulphate of ammonia. However, from 1922 to 1927, inclusive, both tanks had received the same quantity of lime. A comparison of the calcium removals in the crops and drainage water during these years should therefore indicate what effect, if any, sulphate of ammonia exerted on the removal of calcium. These data are shown in table 8, the figures being given for each year separately.

The same tendency noted in table 7 is apparent in table 8.

In every year except the first the removal of calcium in the crops and drainage water was greater from the tanks that received sulphate of ammonia than from those that received nitrate of soda. Moreover, from 1922 to 1925, inclusive, there was little or no change in soil reaction. The evidence of these years indicates that the addition of sulphate of ammonia is in itself enough to cause a greater removal of calcium, although in later years the application of lime may have contributed to the same end.

TABLE 8.—*Calcium removed in crops and in drainage water, during successive years, from tanks receiving sulphate of ammonia or nitrate of soda*

[Data expressed as pounds per acre]

Year	Calcium removed in—					
	Crops from tanks receiving sulphate of ammonia (nos. 21, 24)	Crops from tanks receiving nitrate of soda (nos. 2, 23)	Drainage from tanks receiving sulphate of ammonia (nos. 21, 24)	Drainage from tanks receiving nitrate of soda (nos. 2, 23)	Crops and drainage from tanks receiving sulphate of ammonia (nos. 21, 24)	Crops and drainage from tanks receiving nitrate of soda (nos. 1, 23)
1922	11.9	11.6	294.5	316.2	306.4	327.8
1923	29.9	23.7	208.5	188.7	238.4	212.4
1924	50.8	61.2	383.9	302.2	434.7	363.4
1925	36.0	30.1	312.7	235.0	348.7	265.1
1926	25.7	24.8	631.4	447.4	657.1	472.2
1927	50.8	36.1	521.7	289.4	572.5	325.5

It would naturally be assumed that the concentration of calcium in the drainage water was greater in the flow from the sulphate of ammonia tanks than from the others. Calculations, when weighted for the drainage measurements and for the calcium found in all of the tanks for the period 1922–31 give the calcium concentration for the water from the sulphate of ammonia tanks at 98.5 parts per million and from the nitrate of soda tanks at 72.5 parts per million.

SUMMARY

Six lysimeter tanks each holding $3\frac{1}{2}$ tons of soil were used in an experiment to ascertain whether as much nitrogen was removed in drainage water from a sandy soil fertilized with large quantities of sulphate of ammonia as from the same soil fertilized with an equivalent quantity of nitrate of soda. The experiment was begun in 1922 and concluded in 1931.

At the beginning of the experiment, the annual application of nitrogen amounted to 82.35 pounds per acre. Later, nitrogen was increased to twice that amount. Ground limestone was applied to all tanks at the rate of 2 tons to the acre at the beginning of the experiment and 1 ton in 1926. After 1926 no lime was given the soil receiving nitrate of soda, but the soil receiving sulphate of ammonia was eventually given 8 tons in an effort to prevent it from becoming too acid.

The hydrogen-ion concentration of the soil did not change materially from the beginning of the experiment in 1922 up to 1926. From that time on there was a constant tendency for the soil treated with sulphate of ammonia to become more acid, and to counteract this tendency 5 tons more of limestone were applied to the soil so treated.

The cropping system consisted of spinach planted in the spring, followed by carrots in the summer. After the carrots were harvested, rye was sown, allowed to grow until spring, and then turned under. This cropping was alternated with lettuce planted in the spring, followed by garden beets in the summer, and rye again grown as a green-manure crop.

Determinations of nitrogen and calcium were made in the drainage water; in the crops dry matter, nitrogen, and calcium were determined.

The average quantity of nitrogen removed annually in drainage water during the entire experimental period was approximately 29 pounds to the acre from the soil that received sulphate of ammonia and nearly 39 pounds from the soil that received nitrate of soda.

In the tanks treated with nitrate of soda crop growth was appreciably greater than in those treated with sulphate of ammonia. The crops in the nitrate of soda tanks removed about 108 pounds of nitrogen to the acre annually, and the crops in the sulphate of ammonia tanks removed 96 pounds. The total average annual removal of nitrogen in crop and drainage combined amounted to between 147 and 148 pounds per acre from the nitrate of soda tanks and 125 pounds from the sulphate of ammonia tanks.

From 1922 to 1925, inclusive, the hydrogen-ion concentration of the soil treated with sulphate of ammonia was approximately the same as that of the soil to which nitrate of soda was applied (pH 6.3 to 6.5). During this period the crop yields in the sulphate of ammonia tanks were smaller than in the nitrate of soda tanks, the ratio being 100 to 117. During 1930-31 the pH readings of the soil in these sulphate of ammonia tanks were 6.6 to 7.3 and those in the soil of the nitrate of soda tanks were 7.5 to 8.1. In these years the crops in the soil receiving sulphate of ammonia still yielded less than those in the soil receiving nitrate of soda, the ratio in this case being 100 to 119. There did not appear to be a relatively better growth produced by the sulphate of ammonia when the soil reaction approached neutrality than when it was more decidedly acid.

In the years in which the crop contained a large amount of nitrogen the quantity in the drainage water was small, and conversely, when the crops contained little nitrogen the quantity in the drainage water was large. The largest removal of nitrogen in drainage in any one year amounted to 68 pounds to the acre from the tanks that received sulphate of ammonia and 83 pounds from those that received nitrate of soda.

Although larger crop yields were produced in the soil that received nitrate of soda, these crops contained less calcium both in percentage and by weight than the crops from soil that received sulphate of ammonia. Moreover, the drainage water from the soil receiving ammonium sulphate contained more calcium than that from soil receiving nitrate of soda. This relationship was recorded both for the period 1922-27, when all the tanks received the same quantities of limestone, and for the period 1922-25, when there was little or no difference in soil reaction in any of the tanks. It may therefore be considered to be associated with the effect of the sulphate of ammonia. The average concentration of calcium in the drainage water from the sulphate of ammonia tanks was 98.5 parts per million, and that from the nitrate of soda tanks was 72.5 parts per million.

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VARIABILITY IN THE PEA-WILT ORGANISM,
FUSARIUM ORTHOCERAS VAR. PISI¹

By WILLIAM C. SNYDER,

Formerly research assistant, Wisconsin Agricultural Experiment Station²

INTRODUCTION

The occurrence of host specialization within species of parasitic fungi is widespread, being encountered frequently among the Basidiomycetes, Ascomycetes, and Fungi Imperfecti. In certain of the vascular *Fusaria* specialized strains of the parasite possessing different pathogenic capacities have been noted. Broadfoot (3)³ described nine physiologic forms of the flax-wilt organism, *F. lini* Bolley, on the basis of various degrees of resistance recognized in four different flax varieties. The cotton-wilt fungus, *F. vasinfectum* Atk., was shown by Fahmy (8) to comprise three strains, depending upon whether the source of the fungus was Egypt, India, or North America. In a study of 24 collections of the tomato-wilt pathogene, *F. lycopersici* Sacc., White (20) was able to relegate all strains to two general classes on the basis of pathogenicity. These two groups were exemplified by the type A and B strains further studied for pathogenic and cultural differences by Haymaker (11). The A type was found consistently more pathogenic than the B type, but the difference proved to be a matter of degree rather than of kind, for the variations in strain virulence were uniform on all host varieties tested. Blank⁴ encountered no selective pathogenicity or consistent variation in the degree of virulence of 11 collections of the cabbage-yellows parasite, *F. conglutinans* Wr., when applied to various subspecies of *Brassica oleracea* L.

In the pea-wilt disease, control is founded upon the use of resistant varieties. It is clear, therefore, that a survey of geographic isolations of the wilt fungus, *Fusarium orthoceras* App. & Wr. var. *pisi* Linford, is basic to the further development and use of wilt-resistant pea varieties. With this in mind, a study was made to determine the constancy in selective pathogenicity, and other characters, of the fungus.

PATHOGENICITY OF STRAINS OF THE PEA-WILT FUSARIUM

EXPERIMENTAL METHODS

A preliminary field trial was conducted in 1931 to determine whether a given set of pea varieties would react differently to naturally infested wilt soils in the different pea sections of Wisconsin. No evidence was found that pointed unmistakably to host specialization. A

¹ Received for publication Sept. 22, 1932; issued August, 1933.² The author acknowledges his indebtedness to Dr. J. C. Walker for the valuable suggestions offered during the course of these investigations and for aid in preparation of the manuscript.³ Reference is made by number (italic) to Literature Cited, p. 87.⁴ BLANK, L. M. A STUDY OF VARIATION IN THE CABBAGE YELLOWS ORGANISM, *FUSARIUM CONGLUTINANS* WR. Unpublished thesis, Univ. Wis. 1930.

more comprehensive study in the greenhouse was then undertaken in which cultures of the fungus derived from all available sources were employed.

For the sake of convenience, all isolations of the pea-wilt *Fusarium* studied from the standpoint of differences either in respect to pathogenicity or cultural characters will be termed "strains." In table 1 the regional origin of the fungus strain is indicated in each case by the numeral of the strain symbol, and isolants from the same region by the small letter following. Two or three isolations from each

TABLE 1.—Source of *Fusarium orthoceras* var. *psi* cultures used in the accompanying strain studies

Strain of wilt	Regional source of strain		Material source	Pea-variety source	Date of isolation
	State	Locality			
1a	Washington	Fairfield	(*)	(*)	1929.
1b	do	do	Soil	Perfection	Winter, 1930
2a	Idaho	St. Anthony	Plant specimens	Miscellaneous	Summer, 1930
2b	do	Bonner's Ferry	do	Perfection	Summer, 1931.
2c	do	do	do	Alaska.	Do
3a	Montana	Bozeman	do	Perfection	Do
4a	Wisconsin	Eau Claire	do	do	Summer, 1930
4b	do	Waupun	do	do	Summer, 1929
4c	do	Templeton	do	do	Summer, 1930
5a	Illinois	Rochelle	Soil	do	Winter, 1930.
5b	do	do	do	do	Do
6a	Indiana	Wabash	Plant specimens	Alaska.	Summer, 1931.
7a	Pennsylvania	Hanover	do	Miscellaneous	Do.
7b	Maryland	Silver Run	do	do	Do
7c	do	Westminster	do	do	Do

* Culture WSC 15 received from L. K. Jones

geographic area were selected exhibiting the range of culture type expressed by all isolations from that area. These 15 strains collectively are considered as representative of the pea-wilt fungus as it occurs throughout the pea-growing areas of the United States from Washington to Maryland (fig. 1).⁵

The cultures were purified by repeated transfer on agar plates prior to multiplication upon corn-meal sand. Single-spore cultures were not used because of the possibility of eliminating some potentialities of the original culture and because of the large number of such isolates that would be necessary in order to represent fairly the fungal range in each locality. By using gross cultures of one or more types a good wilt-fungus cross section of a given district could be obtained without the large numbers that would prohibit study of the strains in an experimental unit. Any strain differences observed as a result of these studies might then be further analyzed through the use of monoconidial lines of the fungus.

In the preparation of inoculum, 1-quart Mason jars half filled with a corn-meal sand medium were steam-sterilized and bits of the agar cultures introduced, 8 inoculations being made in this manner from each of the 15 strains. Eleven days later the contents of each jar were incorporated in enough virgin soil of a sandy-loam type to fill a 6-inch pot, and subsequently these were planted to 8 standard pea varieties, 4 of which were susceptible, and 4 resistant to wilt. In

⁵ Since the completion of this manuscript the writer has found the pea-wilt fungus present also in California.

this set-up each strain could be tested simultaneously with 8 pea varieties, and by planting all the pots at one time a direct comparison of the host-parasite reactions of all strains of the fungus and of all pea varieties became possible. This procedure permitted a very heavy and uniform soil inoculation, insuring rapid wilting. Uninoculated check pots occupied the center row for the entire length of the greenhouse bench. Cross inoculation between pots was not a consideration in the experiment because, as has been shown elsewhere (19), appreciable quantities of soil inoculum must be transferred in order to function in disease production before three successive croppings to peas. Moreover, such high concentrations of inoculum were used that any effect of even a deliberate soil transfer would be overshadowed by the

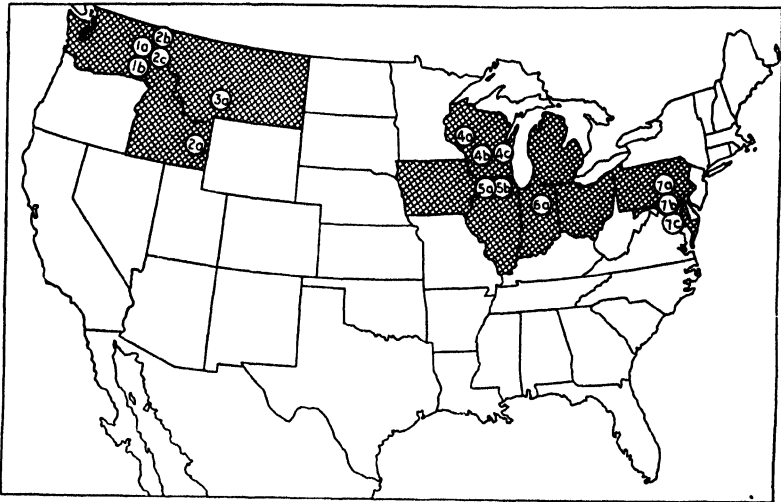


FIGURE 1.--Geographic distribution of the pea-wilt fungus as it occurs in the United States (18). Cross-hatching indicates the States in which the fungus is known to exist, while the circles designate the origin and number of the strains used in connection with the studies recorded here. The fungus was isolated from specimens sent in from Iowa during the summer of 1932.

reaction to the mass culture. The pots were used for only three plantings, and the check row was watched throughout the trials for any evidence of disease.

RESULTS OF PATHOGENICITY TRIALS

Data obtained from two consecutive plantings, series 1 and 2, of these soil inoculations are shown in tables 2 and 3. They show a marked uniformity of disease response dependent upon the pea variety used, regardless of the fungus strain employed. The two discontinuous classes of pea plants in respect to wilt resistance, classified in an earlier study (17) and here maintained when subjected to a wide collection of pathogenic wilt strains, are illustrated in figure 2, A. It will be observed that all the susceptible plants are completely dead and that all the resistant varieties are healthy. A few plants of susceptible Alaska still remain standing, but only one remained at the conclusion of the series. This stock of Alaska was found to have a scattering of wilt-resistant seed in it, not an uncommon experience with the Alaska variety. The percentages of wilt in



FIGURE 2.—A, A comparison of resistant and susceptible varieties from series 1 (table 2) 26 days after planting. All the pots shown here were inoculated with the fungus strain 7b from Maryland. Resistant (R) and susceptible (S) varieties are alternated as follows: *a*, Green Admiral (R); *b*, susceptible Alaska (S); *c*, resistant Alaska (R); *d*, Ashford (S); *e*, Alderman (R); *f*, Thomas Laxton (S); *g*, Bruce (R); *h*, Perfection (S). The plants of susceptible Alaska still standing in the photograph all wilted later except one which proved to be a resistant plant. B, Classification of wilt symptoms used in strain virulence studies: *a*, Healthy plant; *b*, diseased plant in early stages of wilt, showing stunting and recurving of the stipules and leaflets, at which stage the color is usually yellowish green; *c*, diseased plant completely wilted with all the foliage dead and shriveled although the stem is still green and turgid.

the susceptible varieties was smaller as a rule in the first series than in the second. The reason lies in the fact that during the first series of experiments growing conditions were unusually poor, the days were approaching their minimum in point of daylight, and sunshine was almost a rarity. Artificial light was employed but only with partial success. When final readings were taken, 7 weeks after planting, wilting was not complete in all cases, and some plants were collapsing as a result of spindly growth.

TABLE 2.—*Test of pea varieties susceptible and resistant to wilt against 15 different strain inoculations obtained from 7 pea-growing regions of the United States extending from Washington to Maryland*

SUSCEPTIBLE

Strain of wilt	Susceptible Alaska				Ashford			
	Series 1		Series 2		Series 1		Series 2	
	Plants	Wilted	Plants	Wilted	Plants	Wilted	Plants	Wilted
	Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	Number	Percent (%)
1a.....	25		25		22		25	
1b.....	22	86	26	100	23	100	26	100
Control.....	26	0	25	0	23	0	25	0
2a.....	26	81	24	96	21	86	29	100
2b.....	25	84	26	100	22	100	28	100
2c.....	25	80	25	96	22	100	25	100
3a.....	35	63	24	92	33	91	33	100
Control.....	31	0	25	0	27	0	25	0
4a.....	35	91	21	100	36	100	26	100
4b.....	34	62	25	92	29	96	24	100
4c.....	38	79	23	91	26	96	29	100
5a.....	42	83	22	96	30	100	28	100
5b.....	33	82	27	100	35	97	29	100
Control.....	39	0	25	0	24	0	25	0
6a.....	42	83	28	100	34	100	31	100
7a.....	40	100	27	93	36	100	28	100
7b.....	46	96	28	96	35	100	33	100
7c.....	40	95	25	100	31	77	26	100
Control.....	31	0	25	0	27	0	25	0

Strain of wilt	Thomas Laxton				Perfection			
	Series 1		Series 2		Series 1		Series 2	
	Plants	Wilted	Plants	Wilted	Plants	Wilted	Plants	Wilted
	Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	Number	Percent (%)
1a.....	24		28		19		24	
1b.....	25	88	29	100	26	100	26	100
Control.....	21	0	25	0	23	0	25	0
2a.....	20	65	27	100	27	89	30	100
2b.....	25	76	29	100	25	100	27	100
2c.....	24	100	26	100	28	100	29	100
3a.....	27	81	24	100	28	96	27	100
Control.....	26	0	25	0	25	0	25	0
4a.....	27	100	29	100	26	100	26	100
4b.....	28	100	33	100	29	86	28	100
4c.....	35	91	31	100	27	96	25	100
5a.....	37	100	29	100	29	100	28	100
5b.....	38	100	26	100	30	100	31	100
Control.....	29	0	25	0	32	0	25	0
6a.....	32	87	29	100	31	97	28	100
7a.....	35	91	30	100	24	100	28	100
7b.....	36	100	28	100	40	100	29	100
7c.....	35	97	32	100	38	87	34	100
Control.....	35	0	25	0	37	0	25	0

See footnotes at end of table.

TABLE 2.—Test of pea varieties susceptible and resistant to wilt against 15 different strain inoculations obtained from 7 pea-growing regions of the United States extending from Washington to Maryland—Continued

RESISTANT								
Strain of wilt	Green Admiral				Resistant Alaska			
	Series 1		Series 2		Series 1		Series 2	
	Plants	Wilted	Plants	Wilted	Plants	Wilted	Plants	Wilted
	Number	Per-cent	Number	Per-cent	Number	Per-cent	Number	Per-cent
1a.....	20	0	24	0	25	0	26	0
1b.....	20	0	27	0	25	0	27	0
Control.....	22	0	26	0	25	0	24	0
2a.....	21	0	31	0	24	0	28	0
2b.....	21	0	32	0	21	0	33	0
2c.....	20	0	25	0	15	^b 0	30	0
3a.....	27	0	29	0	30	0	27	0
Control.....	31	0	25	0	30	0	25	0
4a.....	30	0	24	0	34	0	29	0
4b.....	32	0	27	0	33	0	33	0
4c.....	39	0	28	0	30	0	32	0
5a.....	33	0	28	^b 0	35	0	30	0
5b.....	33	0	31	^b 0	35	^b 0	28	0
Control.....	27	0	25	0	37	0	25	0
6a.....	31	0	29	0	40	0	26	0
7a.....	29	0	28	^b 0	40	0	24	0
7b.....	33	3	32	3	40	2	31	^b 0
7c.....	31	0	27	^b 0	37	0	30	0
Control.....	29	0	29	0	40	0	28	0

Strain of wilt	Alderman				Bruce			
	Series 1		Series 2		Series 1		Series 2	
	Plants	Wilted	Plants	Wilted	Plants	Wilted	Plants	Wilted
	Number	Per-cent	Number	Per-cent	Number	Per-cent	Number	Per-cent
1a.....	20	0	26	0	25	0	27	0
1b.....	25	0	23	0	27	4	26	4
Control.....	25	0	25	0	25	0	25	0
2a.....	25	0	29	0	26	0	29	0
2b.....	25	0	28	0	28	0	28	0
2c.....	25	0	30	0	29	^b 0	30	0
3a.....	25	0	32	0	31	0	29	0
Control.....	25	0	25	0	25	0	25	0
4a.....	32	0	28	0	29	0	29	0
4b.....	35	0	33	0	31	0	31	0
4c.....	34	0	30	0	27	0	32	0
5a.....	32	0	34	0	32	^b 0	30	3
5b.....	30	0	27	0	31	^b 0	33	9
Control.....	25	0	25	0	25	0	29	0
6a.....	32	0	29	0	27	0	30	0
7a.....	35	0	30	0	33	^b 0	28	^b 0
7b.....	39	0	41	0	30	^b 0	31	6
7c.....	40	0	33	0	34	0	29	0
Control.....	38	0	28	0	30	0	27	0

^a Incomplete. Owing to the slow rate of wilting obtained with this strain the percentage of wilt at the completion of both series was not comparable to other data in the table, so was not recorded.

^b Leaf symptoms, usually on 1 to 5 plants, suggested wilt, but culture of the plants on agar did not yield the fungus.

Upon the basis of these data it appears that the programs of selection and breeding for pea varieties resistant to *Fusarium orthoceras* var. *pisi* are built upon the relatively firm foundation of (1) the stable nature of the wilt pathogene as regards kind of pathogenicity, and (2) the hereditary nature of resistance, carried by a single dominant

factor (17). These facts make possible a simple and very effective means of pea-wilt control. On the other hand, however, the possibility remains that a differentially pathogenic strain of the parasite may sometime appear, or may even now occur undetected. The wide differences between strains in respect to other fungal characters emphasizes this possibility.

TABLE 3.—Summary of resistance to 14 strains of the *Fusarium wilt fungus* of 8 pea varieties planted in series 2 (table 2) on artificially inoculated soil, as compared to a planting from the same seed stocks on naturally infested Waupun wilt soil

Pea variety	Artificially inoculated soil		Naturally infested Waupun soil	
	Plants	Resistance	Plants	Resistance
	Number ^a	Percent ^b	Number	Percent
Green Admiral	398	99.7	91	100
Resistant Alaska	408	100	115	99.2
Alderman	427	100	52	100
Bruce	415	98.4	124	100
Susceptible Alaska	356	3.3	122	8.9
Ashford	395	0	93	2.1
Thomas Laxton	399	0	106	1.9
Perfection	396	0	127	.8

^a These numbers do not include those planted on the soils inoculated with fungus 1a

^b Based upon the number of instances of susceptibility or resistance encountered throughout the experiment with each variety.

COMPARATIVE VIRULENCE OF STRAINS

Although no evidence was obtained in the course of these investigations to suggest that a differential in host specialization between strains exists, it appeared that the strains varied somewhat in respect to virulence or degree of pathogenicity. Records on the appearance of symptoms and dates at which complete wilting or death of the plants occurred showed significant differences in this regard. A comparison was possible, owing to the fact that all pots in the experiment had received identical treatment. In the second series, those pots in which the resistant varieties had grown during the first series, were planted with susceptible seed and vice versa. By the end of the second series it was expected any minor differences in inoculum concentration of the soils had become stabilized through the intensive culture of peas. An additional planting at this time was planned in order to record differences in strains in respect to rate of wilting using only one variety of pea, and to determine whether these differences corresponded with those noted in the previous series.

The pots were planted with Perfection seed, and the total stand in each case was taken. Every 2 days counts were made of the number of plants showing symptoms, and of the number dead, i.e., those plants whose foliage was entirely wilted and shriveled. These two symptom classes, distinct in themselves, are shown in figure 2, B. In table 4 are computed the percentages of plants diseased, and dead, at 2-day intervals following planting. It is seen that the variation in appearance of symptoms ranges from 14 days after planting in several strains to 28 days in the case of 1a. Similarly, complete wilting is an index of the relative degree of strain pathogenicity. In the last column the strains are rated in respect to virulence both as indexed by

symptom appearance and complete wilting. At the top of the column are the most virulent strains, and at the bottom the least virulent ones. By plotting the percentages, either of diseased or of dead plants against time in days, curves representing the relative virulence of each strain were found to parallel each other closely in form and type and to fall into four general groups. Since parallelism of the curves was most pronounced in the region of the graph representing 50 percent killing of plants, a comparative rating of strains was made at this point. Accordingly, the value in days for each curve as it crossed the 50 percent line was read on the graph, and these figures were used in constructing figure 3. All strains are seen to fall into four groups, the first three of which are separated by approximately 3-day intervals, and a fourth group represented by strain 1a alone. Observations in both series 1 and 2, in conjunction with this experiment,

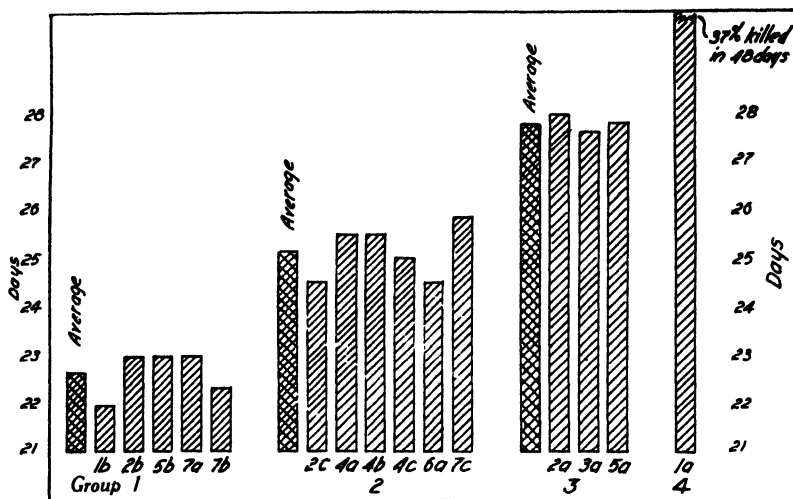


FIGURE 3.- Comparison of the number of days required for each wilt strain to kill 50 percent of the plants.

support the arbitrary classification portrayed here. In any event it is clear that differences occur between strains. Strains 1b and 7b in all inoculation work have always been in the most virulent class. Strain 1a has been the most divergent of all the strains studied. At the close of the experiment 7 weeks after planting only 37 percent of the plants had been killed. Reisolation of the strain from these plants and reinoculation of soil therewith have given the same type of results, namely, that in spite of a heavy soil inoculation wilting may be completed only in two or three times the interval observed in the case of other strains. Cultural characters also diverge somewhat from the general type range of the strain collection taken as a whole.

In order to compare the strains under more closely similar conditions the fungus was reisolated from diseased plants on soils 1a, 2a, 2b, 2c, 4c, and 7c, these representing the four groups shown in figure 3. Isolations were all made at the same time, multiplied on the same weight of corn-meal sand, and equal volumes of soil inoculated and planted the same day. In this manner each strain was recovered in a pathogenic state, and maintained in artificial culture for the same

period of multiplication before being returned to a new lot of thoroughly mixed untreated virgin soil. The interval between the recovery of the strain from diseased plants and reincorporation in the soil, was 18 days. The relative rate of wilting obtained was of the same order as indicated in figure 3, except that 2a took a position between 2b and the group 2c, 4c, and 7c.

TABLE 4.—Rate of appearance of wilt symptoms, and of death, as expressed in percentages of affected Perfection pea plants during a period of days, for different cultural strains of the wilt fungus

Strain of wilt	Plants	14 days		16 days		18 days		20 days		22 days		24 days		26 days	
		Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead
	Number	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1a	140	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1b	121	4.1	0	97	0	98.4	0	100	36.4	100	49.6	100	79.3	100	96
2a	148	0	0	0	0	4.1	0	16.2	0	47.3	0	69	7.4	90	21
2b	184	2.8	0	90.2	0	92	0	97.3	15.2	100	34.2	100	74	100	96.2
2c	46	0	0	2.2	0	74	0	75	4.3	85	8.7	100	32.6	100	87
3a	211	0	0	0	0	2.4	0	10.9	0.5	64	1.4	84	2.8	96	10.9
4a	189	0	0	1.1	0	2.6	0	93	0	96	1.1	98	7.9	100	73
4b	247	0	0	2.0	0	3.6	0	94	0	96	0.4	98	8.9	100	70
4c	225	23.2	0	89	0	90	0	91	4.5	93	5.8	96	9.3	99	84
5a	56	0	0	0	0	3.6	0	16	0	53.6	0	89	3.6	100	14.3
5b	208	0	0	2.9	0	91	0	100	8.6	100	28	100	78	100	93
6a	201	2.5	0	85	0	90	0	91	6.9	91	11	97	40	99	67
7a	68	1.5	0	85	0	90	0	94	13.2	94	30.9	98.5	72	98.5	89.8
7b	76	92	0	96	0	96	0	100	10.5	100	42	100	77.7	100	97.5
7c	279	0	0	0	0	1.1	0	12.2	0	54	0	65	35.8	87	51

Strain of wilt	Plants	28 days		30 days		32 days		40 days		48 days		Order of rating of wilt strains *	
		Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	By disease	By death
	Number	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent		
1a	140	100	100	100	100	100	100	100	100	100	100	7b	7b
1b	121	100	100	100	100	100	100	100	100	100	100	1b	1b
2a	148	100	57.4	100	68	100	82	100	100	100	100	7a	7a
2b	184	100	99	100	100	100	100	100	100	100	100	4c	2b
2c	46	100	100	100	100	100	100	100	100	100	100	2b	5b
3a	211	100	54	100	100	100	86	100	100	100	100	6a	6a
4a	189	100	95	100	100	100	100	100	100	100	100	5b	2c
4b	247	100	95	100	100	100	99	100	100	100	100	2c	4c
4c	225	100	93	100	100	100	98	100	100	100	100	4b	4b
5a	56	100	57	100	100	100	77	100	100	100	100	4a	4a
5b	208	100	98	100	100	100	100	100	100	100	100	3a	7c
6a	201	100	91	100	100	100	96	100	100	100	100	2a	3a
7a	68	100	95.6	100	100	100	100	100	100	100	100	7c	2a
7b	76	100	98.8	100	100	100	100	100	100	100	100	5a	5a
7c	279	100	66	100	100	100	86	100	100	100	100	1a	1a

* Listed in descending order of virulence.

A comparison was next made with single-spore cultures from several strains, as follows: 1a5, 2a2, 4b1, 4c4, 5b3, and 7b5. They were used in duplicate, multiplied on weighed amounts of inoculum, and mixed into the soil in the same manner as before. The single-spore lines of 5b and 7b fell into the most virulent group, 4b and 4c were of inter-

mediate virulence, and 1a was the least virulent. Strain 2a again approached in pathogenicity group 1.

The results up to this point argued for a general constancy of virulence for a given strain culture. However, the experience with strain 2a, indicating that different cultures of the same strain source might vary in degree of pathogenicity, and the additional fact that isolations from the same geographic regions may exhibit all the degrees of virulence illustrated in figure 3, suggested the possibility that a given strain might be made to yield forms both higher and lower in virulence than the original. In order to test this possibility a sus-

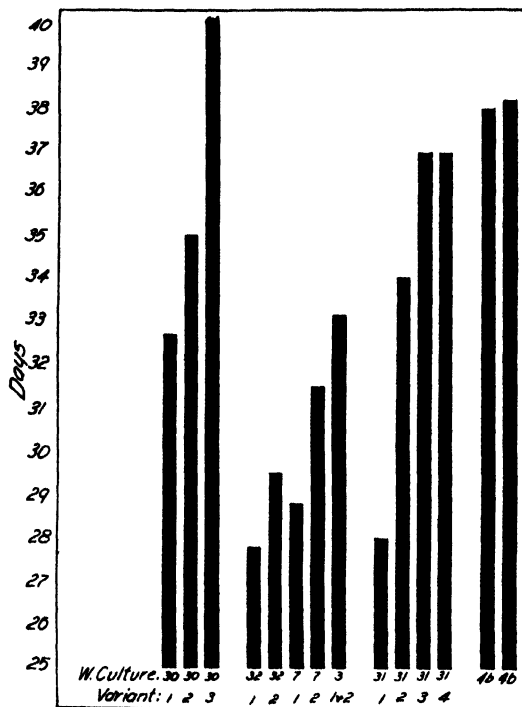


FIGURE 4.—Comparison of the number of days required to obtain 50 percent killing of plants with segment cultures (variants) of several isolations of the wilt fungus from the same source

ceptible variety of peas was grown upon naturally infested Waupun soil, the original source of strain 4b. Thirty-five resulting wilted plants were cultured and 35 isolations of the wilt fungus obtained. Single-plate colonies were made of each isolation for observation in respect to purity, and they were then transferred, one colony per plate, to 5 percent potato-dextrose agar, a medium found favorable for the separation of variants. Five of the cultures were conspicuous in the production of cultural types; these were built up on cornmeal sand and aliquots of virgin soil inoculated and planted. The results are shown in figure 4. Comparison with figure 3 is not possible, because this series was grown at a time when for 2 or 3 weeks there was but little sunshine and wilting proceeded slowly. However, interrelationships were not altered, and two inoculations with the original stock culture 4b were run for a standard.

It is realized that the use of segment cultures from a gross isolation of the fungus in an endeavor to demonstrate variability within a single colony of the fungus is open to the objection that the original isolation may have contained two or more strains. It became essential, therefore, in considering the potentialities of a single thallus in producing variants with different capabilities in regard to virulence, to run an experiment with single-spore cultures and compare the virulence of variants obtained from them, both in relation to each other and to the parent culture. Culture 4b was selected for this

experiment. A susceptible variety of peas was grown upon naturally infested Waupun soil, the original source of strain 4b. Thirty-five resulting wilted plants were cultured and 35 isolations of the wilt fungus obtained. Single-plate colonies were made of each isolation for observation in respect to purity, and they were then transferred, one colony per plate, to 5 percent potato-dextrose agar, a medium found favorable for the separation of variants. Five of the cultures were conspicuous in the production of cultural types; these were built up on cornmeal sand and aliquots of virgin soil inoculated and planted. The results are shown in figure 4. Comparison with figure 3 is not possible, because this

purpose. The procedure consisted in placing several monoconidial lines of strain 4b upon a medium which readily permitted the detection of variants. Mycelial transfers of the sectors were then made from time to time. Two of these variants are illustrated in figure 5. In this way a collection of variants was obtained, all of which originated in single-spore cultures of one strain. Several of the more stable of these dissociants were then multiplied on equal weights of corn-meal sand and incorporated in aliquots of soil under identical conditions. Essentially the same relative virulence was obtained as with segments of gross cultures of the strain. The dissociants possessed a virulence higher, lower, or the same as the monoconidial parent culture. These relative differences are shown in figure 6.

The foregoing experiments established the fact that within the fungus causing the Fusarium wilt of pea there occur strains differing in degree of pathogenicity. The differences in virulence obtained were

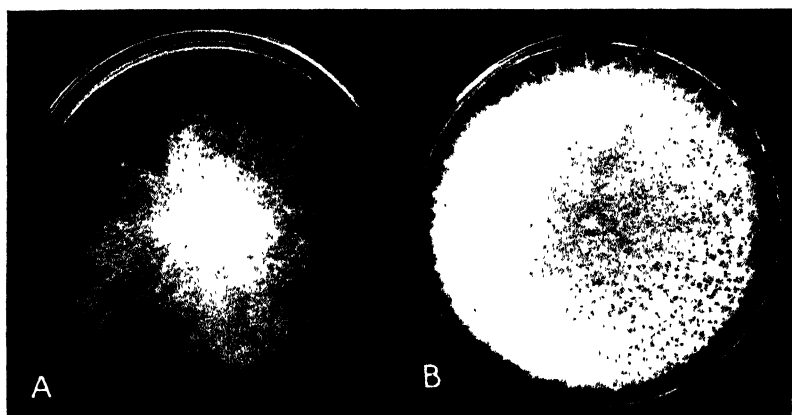


FIGURE 5.—Two segment cultures from the same parent single-spore colony (4b5). A produced a deep red pigmentation in the medium, B exhibits more abundant aerial mycelium, more rapid radial growth, and lacks pigmentation

not wide as a general rule, but they were clearly defined. It is, of course, not to be expected that a variable fungus, existing in a labile state, should exhibit fixed pathogenicity relationships, especially where fragment cultures representative of the most unstable condition of the fungus are compared.

Repeated inoculations with the same stock culture have yielded results all of the same order. For example, a dozen soil inoculations made over a period of a year with stock culture 4b have always exhibited an intermediate type of virulence, and the same is true of other strains. However, one geographic region or even a locality is not to be considered inhabited by a single strain type. Two widely divergent strains have been studied from the same pea region in Washington. Attempts to build up the virulence of the weaker strain (1a) by repeated reisolation and reinoculation have failed. The strains from Maryland on the opposite coast of the United States showed similar differences in degree of pathogenicity and so with other regions. Strains both more and less virulent than the original 4b culture were obtained from the same bed of Wisconsin soil, using monoconidial lines. Thus a locality or region may be dominated by

a strain type but not restricted to that type. This is to be expected in view of the dissociating nature of the fungus, and the fact that it is seed borne (16).

Duplicate inoculations of all trials have been practically identical in their pathogenesis, thereby vouching for the technic employed.

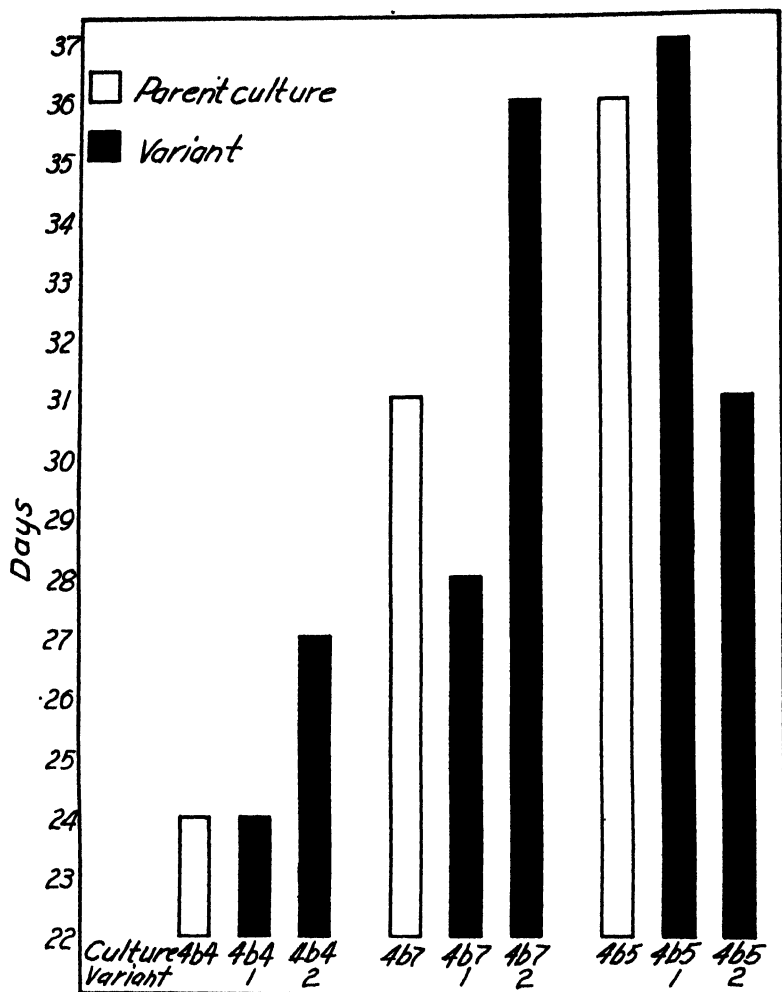


FIGURE 6. -Comparison of the number of days required to obtain a 50 percent killing of plants with segment cultures (variants) from monoconidial parent cultures of wilt strain 4b.

It has occurred to the writer that although equal amounts of corn-meal sand were inoculated with each strain, different growth rates upon the medium might result in different amounts of fungus being added to the soil, and that the greater virulence was a direct correlation of a higher concentration of fungus inoculum and not an expression of fungal potency. Cultural characteristics of the strains are discussed elsewhere; it suffices to state here that no direct correlation has been encountered between virulence and growth, in fact

some of the fastest and most profuse growers possess the least virulence. Correlation with other cultural characters are discussed later in this paper.

PHYSIOLOGY OF THE PEA-WILT FUSARIUM

In the original description of *Fusarium orthoceras* var. *pisi* it was noted that cultures of the fungus showed divergence among themselves especially in respect to pigmentation. In fact one agreed closely with *F. conglutinans*, the cabbage-yellows organism (14). Linford, however, reports no comparative study of different isolations. It was deemed opportune, therefore, to parallel the trials of comparative pathogenicities with some observations upon culture characters of the strains.

At the outset it was realized that the building up of a mass of data upon characters of growth in artificial culture could only have limited value in the absolute sense because of the inconstancy of colony type, even where single-spore lines of the fungus were employed. It was hoped, however, to bring out relative differences between strains which might be correlated with results from the pathogenicity trials.

The strains varied somewhat among themselves in respect to several cultural characters. Perhaps the most conspicuous of these was variability in the capacity for the production of color. Differences in type of mycelial growth, whether flat and sparse, or aerial and abundant, were encountered as well as differences in colony margin, rates of growth, staling, and sporulation. Because of the inconstancy of characters within a given stock culture of a strain, it seemed desirable, if not necessary, to confine the study to single-spore lines. Accordingly, a number of monoconidial lines were obtained for each strain. Most of these in each case were of the gross parent type, while others diverged from the original in colony characters. Representatives of the majority type (in every case these were also representative of the original culture) were used in the comparison of strains recorded in table 5. Reisolates from the pathogenicity trials, discussed earlier in this paper, conformed in general to these types, particularly in respect to pigmentation.

TABLE 5.—Comparison of the cultural characters of single-spore lines of the 15 strains of the wilt fungus, grown in Petri dishes upon aliquots of 5 percent potato-dextrose agar

[All data was taken 35 days following inoculation]

Strain of wilt	Sectoring ^a	Aerial mycelium	Pigmentation of—	
			Medium	Hyphae
1a.....	—	Scant.....	White.....	White.
1b.....	—	Abundant.....	Auburn ^b	Do.
2a.....	+	Moderate.....	Dark Perilla purple.....	Maroon.
2b.....	+	do.....	Dark Corinthian purple.....	White.
2c.....	++	do.....	do.....	Do.
3a.....	+	do.....	Walnut brown.....	Do.
4a.....	+	do.....	Dark Perilla purple.....	Do.
4b.....	+	do.....	do.....	Do.
4c.....	—	Abundant.....	Ecu drab.....	Do.
5a.....	—	Moderate.....	Hessian brown.....	Gray-white.
5b.....	—	Abundant.....	Light pinkish cinnamon.....	White.
6a.....	—	Moderate.....	Dark Corinthian purple.....	Do.
7a.....	+	Abundant.....	Corinthian red purple.....	Do.
7b.....	+	do.....	Dark Corinthian purple.....	Do.
7c.....	+	Moderate.....	do.....	Do.

^a (—) indicates no conspicuous sectoring, (+) slight, and (++) moderate.

^b RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D.C. 1912.

Six single-spore lines from 1a, 2a, 4b, 4c, 5b, and 7b, typifying the types most divergent as to cultural and virulent qualities, were selected for further comparisons. A study of these on modified Czapek's medium, using glucose and pectin, respectively, as sources of carbon, was made at five constant temperatures. The results are represented in figure 7. Since the curve for 4c so closely coincided with that for 4b no attempt was made to differentiate between them on the chart. Distinct differences in the rates of growth are illustrated between such

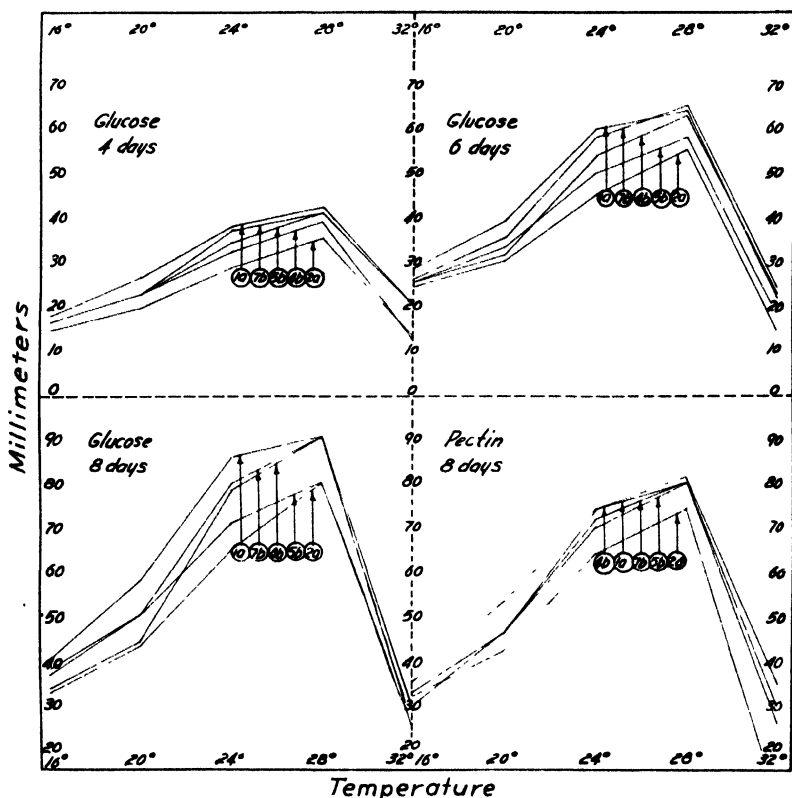


FIGURE 7—Average colony diameters of a monoconidial line from each of the five strains of *Fusarium orthoceras* var. *pin* indicated, at five constant temperatures. A modification of Czapek's medium was employed, using glucose and pectin, respectively, as sources of carbon.

extremes as 1a and 2a, depending upon the medium, but the temperature characteristics reported by Linford (14) in his original description of the fungus were found to hold in general for all types. As a result no wide differences in strain-disease relationships are to be expected in regard to temperature.

These six monoconidial lines and the organisms causing *Fusarium* wilts of aster (*F. conglutinans* var. *callistephi* Beach), beet (*F. conglutinans* var. *betae* Stewart), celery (*Fusarium* sp.), cowpea (*F. tracheiphilum* (E. F. S.) Wr.), and watermelon (*F. niveum* E. F. S.), were used in other comparative studies. The cabbage-yellows pathogene (*F. conglutinans* Wr.) was also included, but a portion of the results were discarded because of bacterial contamination. This fungus

however has in other series agreed closely in reaction with its varieties. A modified Czapek's medium was used.⁵ To this a carbohydrate was added in sufficient quantity to give a final concentration of 5 percent. The solution of the carbohydrate was sterilized separately in the autoclave and added to the medium at the time the plates were poured. At the same time the reaction of the final medium was adjusted to pH 6 except in the case of xylose, xylan, and pectin. All Petri dishes were poured to an approximate depth of three fourths of a centimeter to eliminate the growth differences sometimes experienced on thin agar plates (4), and inoculated in duplicate. Comparable bits of aerial mycelium were taken for this purpose from cultures of the same age on the same medium and the series run at a temperature of 21.5° to 22.5° C. for a period of 5 weeks.

The very close similarity of duplicate cultures throughout the experiment served to emphasize the significance of strain and carbon-source differences. Within a week after the media were inoculated striking differences in pigmentation became apparent. Color as expressed in this series varied in three important respects, namely, (1) rate of appearance, (2) intensity, and (3) shade. Table 6 shows the relative intensity and rate of color formation at the end of 10 and 30 days. Based upon results up to the time the fungus growth had completely filled the Petri dish, the fungi could be separated into two distinct groups, the pigment producers and nonpigment types. At all times differences in relative intensity as well as in shade of color were apparent between strains of the pea-wilt *Fusarium*, for example 2a, 4b, and 7b.

TABLE 6.—Comparison of the ability of certain vascular *Fusaria* to produce pigmentation upon a synthetic agar medium in 10 and 30 days, employing 5 percent concentrations of different carbon sources

Fungus culture	Item	Color of medium as indicated					
		Xylose	Arabinose	Araban	Pectin	Galactose	Glucose
<i>F. orthoceras</i> var. <i>pisi</i> 1a5	10 days	0	0	0	0	1	0
	30 days	Trace	1	Trace	0	2	1
	Quality	202	7L1	8A5	0	56L12	6L12
<i>F. orthoceras</i> var. <i>pisi</i> 2b2	10 days	0	2	0	0	1	0
	30 days	1	3	1	0	1	2
	Quality	3H9	56L6	8J9	0	56L6	56L12
<i>F. orthoceras</i> var. <i>pisi</i> 4b1	10 days	0	0	0	0	0	1
	30 days	0	2	0	0	2	2
	Quality	0	6K11	0	0	7L4	56L12
<i>F. orthoceras</i> var. <i>pisi</i> 4c4	10 days	0	0	0	0	0	0
	30 days	1	2	0	0	1	1
	Quality	2D2	7L1	0	0	7L4	(^b)
<i>F. orthoceras</i> var. <i>pisi</i> 5b3	10 days	0	0	0	0	0	0
	30 days	0	2	0	0	2	1
	Quality	3F10	6011	0	0	5J12	5J12
<i>F. orthoceras</i> var. <i>pisi</i> 7b5	10 days	0	2	1	0	1	2
	30 days	0	3	1	0	3	3
	Quality	2E8	56L12	8A5	0	56L6	56L6
<i>F. conglutinans</i> var. <i>callistephi</i>	10 days	0	0	0	0	0	0
	30 days	0	1	0	0	0	0
	Quality	0	5H9	0	0	0	0
<i>F. conglutinans</i> var. <i>betae</i>	10 days	0	0	0	0	0	0
	30 days	0	0	0	0	0	0
	Quality	0	0	0	0	0	0
<i>Fusarium</i> sp. causing celery wilt.	10 days	1	-----	-----	0	2	1
	30 days	1	-----	Trace	0	2	2
	Quality	-----	-----	-----	0	47L11	5R4
<i>F. tracheiphilum</i>	10 days	2	0	0	0	2	2
	30 days	2	1	0	0	2	2
	Quality	-----	46J5	0	0	47L11	56L8
<i>F. niceum</i>	10 days	0	-----	0	0	1	1
	30 days	0	-----	Trace	0	1	1
	Quality	-----	-----	-----	0	46J7	46J7

^a The numerals indicate a measure of relative intensity; for "quality" see Maerz, A., and Paul, M. R. (15).

^b Lost.

^c MgSO₄, 0.5 g; K₂H₂PO₄, 1 g; KCl, 0.5 g; FeSO₄, trace; asparagine, 1 g; agar, 20 g; H₂O, 1 liter.

TABLE 6.—Comparison of the ability of certain vascular *Fusaria* to produce pigmentation upon a synthetic agar medium in 10 and 30 days employing 5 percent concentrations of different carbon sources—Continued

Fungus culture	Item	Color of medium as indicated				
		Dextrin	Lactose	Saccharose	Maltose	Mannite
<i>F. orthoceras</i> var <i>pisi</i> 1a5	10 days	0	0	0	1	0
	30 days	2	0	2	3	3
	Quality	56L12	0	6L12	56L12	56L12
<i>F. orthoceras</i> var <i>pisi</i> 2b2	10 days	2	0	1	3	3
	30 days	4	Trace	3	4	4
	Quality	56L6	0	56L12	56L6	56L6
<i>F. orthoceras</i> var. <i>pisi</i> 4b1	10 days	1	0	1	0	2
	30 days	3	0	2	2	3
	Quality	56L12	0	56L12	5J11	56L12
<i>F. orthoceras</i> var <i>pisi</i> 4c4	10 days	0	0	0	0	0
	30 days	1	1	2	2	3
	Quality	6B11	5B8	7L6	7L1	56L12
<i>F. orthoceras</i> var <i>pisi</i> 5b3	10 days	0	0	0	0	0
	30 days	1	0	2	Trace.	3
	Quality	6B11	0	6L11	4E10	7L4
<i>F. orthoceras</i> var <i>pisi</i> 7b5	10 days	3	0	2	2	3
	30 days	4	Trace	3	3	4
	Quality	56L6	6A10	56L12	56L12	56L6
<i>F. conglutinans</i> var <i>callistephi</i>	10 days	0	0	0	0	0
	30 days	Trace	0	0	1	3
	Quality	46D1	0	0	5H9	7L1
<i>F. conglutinans</i> var. <i>betae</i>	10 days	0	0	0	0	0
	30 days	0	0	Trace	0	0
	Quality	0	0	0	0	0
<i>varium</i> sp. causing celery wilt.	10 days	3	0	2	1	3
	30 days	3	0	2	2	3
	Quality	56C8	0	5H4	5H4	(b) 3
<i>F. tracheiphilum</i>	10 days	3	0	3	1	1
	30 days	3	Trace	3	2	3
	Quality	47L11	41D2	47L11	46J5	56C8
<i>F. nivum</i>	10 days	1	0	0	1	1
	30 days	1	Trace	1	1	2
	Quality	46J7	46J11	46J7	46J7	(b)

* The numerals indicate a measure of relative intensity, for "quality" see Maerz, A., and Paul, M. R. (15).

^b Lost.

An examination was made of coloration in the steles of wilted plants grown upon the different strain inoculations to see if any relation was borne out between pigment production on artificial media and that where natural vascular discoloration occurred in diseased plants. There seemed to be no rule in this respect for the plants examined, either as to intensity or quality of color.

Attempts were also made to correlate pigmentation and virulence, since culture studies exhibited such pronounced differences in respect to the former. Strain 1a, of lowest virulence, produced no pigment as a rule except after long periods of time on rich carbohydrate media; 7b, exhibiting as high virulence as any strain collected, vied with 2a in rapidity and intensity of pigmentation; 2a exhibited both low and high virulence in various tests, but the single-spore line used here proved to possess a virulence ranking with 7b; 4c, intermediate in degree of virulence, was a poor pigment former, whereas 4b of a similar virulence rating yielded pigment more rapidly than 4c or 1a, but slower than 2a or 7b; 5b failed most conspicuously in the attempted correlation, but both Illinois strains, 5a and 5b, were also markedly different from all the other strains of the fungus in the shade of color when pigmentation did occur. On these high-sugar media the color with all strains was usually of varying intensities of an orange to purple red, except for 5a and 5b which were on the order of a yellow to pink, light brown. When the strains are grouped into classes

according to rate of pigment formation, some manner of correlation between pigmentation and high virulence may be noted in a general way. However, pathogenicity trials in which color and noncolor variants of the same single-spore parent were compared, yielded no consistent data. The extremes in pigmentation of comparable cultures from a very dark almost-black maroon to pure white gave the impression at times that different species rather than strains of a single variety were being studied. All the intensities (though not shades) of pigmentation found between the vascular *Fusaria* cultured were surpassed by the range of intensities within *F. orthoceras* var. *pisi*.

It has been observed (4) that the pH value as well as a high carbohydrate content of the medium, may influence pigmentation. At the conclusion of this experiment a rough approximation of the pH value was obtained by applying a drop of indicator to the agar of the Petri dish. Where pigmentation prevented a direct reading in this manner, a small piece of white filter paper was first pressed upon the agar and the indicator placed upon the paper. With all six strains and on all carbon sources used except pectin, the final pH value was found to be in the neighborhood of 3 to 5. The pectin medium, on the other hand, although most acid to start with, ended in every case on the alkaline side with a pH value of about 8. No pigment was formed in this medium, the light-brown color imparted by the pectin persisting throughout. Whether pigmentation in the cases of strains 2a and 7b could be correlated with a more rapid development of acidity in the medium is not known inasmuch as the pH readings were taken only at the beginning and conclusion of the experiment.

The fungi other than *Fusarium orthoceras* var. *pisi* compared similarly among themselves and with the pea-wilt fungus in most respects. The parasites of cowpea and celery, and next, watermelon, produced more pigment than did the varieties of *F. conglutinans*, but no more than certain strains of the pea fungus. Pigmentation of these species tended to be more on the purple than on the red side. The pH values were also of the same order in most instances where read.

Measurements of radial growth were taken on all plates at 2-day intervals. Duplicate plates showed marked uniformity, for the most part, not varying more than 1 or 2 mm in diameter. Differences in rate of growth between some strains of the pea-wilt fungus were exaggerated on certain carbon sources, as, for instance, on galactose, while in other cases, exemplified by the pectin medium, the differences were small, not exceeding 10 mm of diameter growth. The relative rates of growth varied somewhat with the medium, but strains 1a and 7b were generally the most rapid in radial growth, while 2a and 4b were usually among the slowest. Greatest radial growths of any of the fungi studied were exhibited by the celery and cowpea parasites.

The relative amounts of growth often bore no correlation to radial development of the mycelium. Because of the lack of direct means of comparing cultures in this respect, an arbitrary standard of classification was set up through the use of plate colonies representative of 10 classes of growth occurring in the experiment. Although the basis for the 10 classes is largely an evaluation of abundance of aerial

mycelium, the density and other growth characters have also been taken into account. All colonies were compared in this manner after 10 days and at the end of 30 days. Inasmuch as the ratings were relatively constant throughout the period of growth, only those recorded after 30 days are given in table 7. Strains of the pea *Fusarium*, it is noted, produced the least growth as a rule on xylose, xylan, and arabinose, exhibited various degrees of preference for araban, galactose, glucose, dextrin and sucrose, and did uniformly well on pectin, lactose, maltose, and mannite. Figure 8, photographed at the time the observations in table 7 were recorded, shows all the fungi used in the experiments grown on a medium containing sucrose as the carbon source. Differences in growth between the fungi are evident, but these differences for the most part are no greater than those within the strain range of the pea-wilt fungus.

TABLE 7.—Comparison of the relative amounts of growth produced by certain vascular *Fusaria* in 30 days upon a synthetic-agar medium employing 5 percent concentrations of different carbon sources

[Three fourths of a centimeter thickness of agar was used in each plate and the temperatures maintained at 21.5° to 22.5° C.]

Fungus culture	Growth on indicated medium as evaluated by an arbitrary numerical standard										
	Xylose	Xylan	Arabinose	Araban	Pectin	Galactose	Glucose	Dextrin	Lactose	Sucrose	Maltose
<i>F. orthoceras</i> var. <i>pisu</i> 1a5.....	1	2	2	8	9	5	2	7	9	2	7
<i>F. orthoceras</i> var. <i>pisu</i> 2a2.....	1	2	2	2	6	4	2	3	8	2	5
<i>F. orthoceras</i> var. <i>pisu</i> 4b1.....	2	2	6	8	7	7	4	7	7	4	5
<i>F. orthoceras</i> var. <i>pisu</i> 4c4.....	2	3	6	8	7	7	6	2	8	8	8
<i>F. orthoceras</i> var. <i>pisu</i> 5b3.....	1	2	6	3	7	8	6	6	7	8	7
<i>F. orthoceras</i> var. <i>pisu</i> 7b5.....	2	4	7	8	8	3	6	7	7	6	7
<i>F. conglutinans</i> var. <i>cultristephi</i>	1	1	2	1	4	3	2	3	2	2	2
<i>F. conglutinans</i> var. <i>betar</i>	1	1	2	2	7	7	3	3	6	3	3
<i>Fusarium</i> sp. causing celery wilt.....	4	4	2	2	2	2	10	3	10	10	6
<i>F. trochetaephilum</i>	4	4	2	3	9	7	10	6	10	10	3
<i>F. nigrum</i>	3	3	3	3	6	6	4	4	6	7	2

In comparing the growth characters the phenomenon of spiral colony development was observed in many cases, especially during the early stages of growth. Where present, the spiral was recorded as having either a clockwise or counterclockwise direction. This feature was particularly conspicuous upon lactose and xylan, and moderately so on mannite, pectin, galactose, and maltose. A record of the phenomenon in these cases is found in table 8.

TABLE 8.—Occurrence and direction of spiral colony development of monoconidial lines of the pea-wilt fungus upon different carbon sources

[C indicates clockwise and A counterclockwise growth]

Strain of wilt	Growth on type of medium indicated							
	Lactose	Xylan	Maltose	Galactose	Pectin	Mannite	Arabinose	Araban
1a5.....	C	---	---	A	C	C	C	C
2a2.....	C	C	C	A	C	C	C	C
4b1.....	C	---	C	A	---	C	A	---
4c4.....	C	C	C	A	C	C	C	C
5b3.....	C	C	C	---	C	---	---	C
7b5.....	---	C	C	A	C	C	A	---

There appears to be uniformity among these single-spore lines in regard to the direction of spiraling, but this uniformity was not observed in the various other fungal species and varieties in the experi-

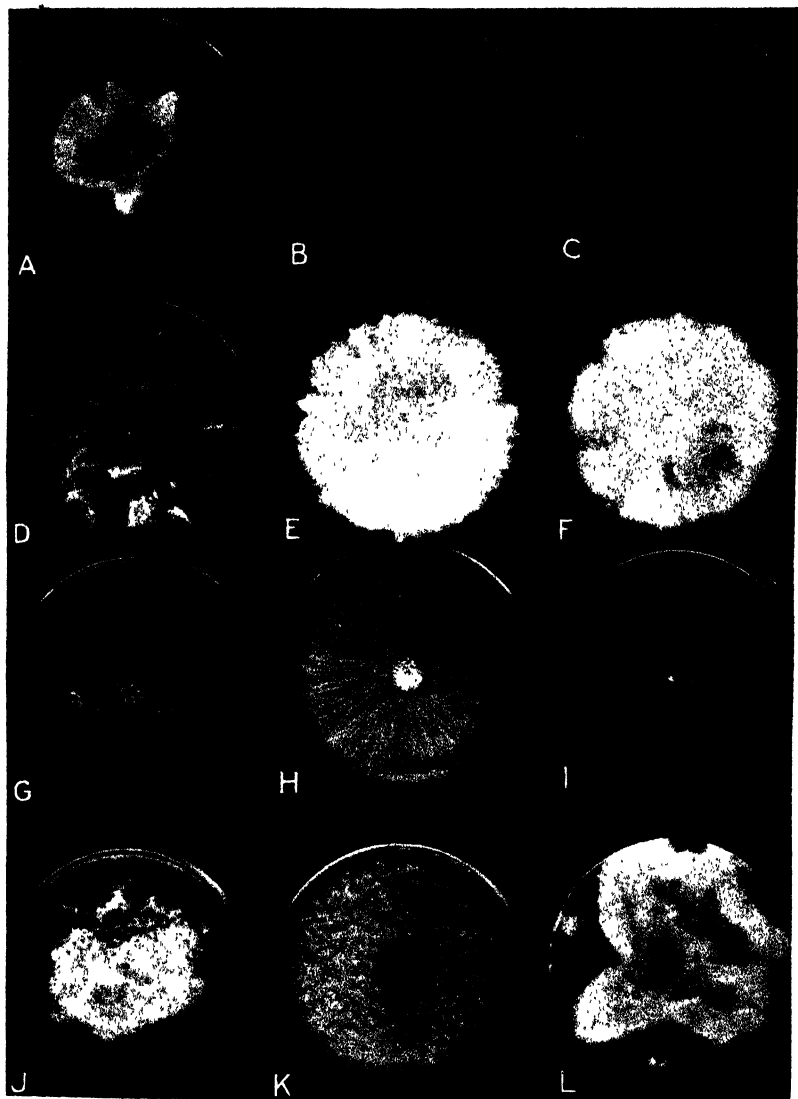


FIGURE 8.—Vascular *Fusaria* grown on a modification of Czapek's medium, containing sucrose as a source of carbon: A, 4b1; B, 2a2; C, 1a5; D, 7b5; E, 5b3; F, 4c4. All these are monoconidial cultures of *F. orthoceras* var. *pisi*. G, *F. conglutinans* var. *betae*; H, *F. conglutinans* var. *callistephi*; I, *F. conglutinans*; J, *F. niveum*; K, *F. tracheophilum*; L, *Fusarium* sp. from celery. Note the range of colony character among the strains of *F. orthoceras* var. *pisi*, in comparison with the range of all the vascular *Fusaria* shown.

ment, regardless of the carbon source. The wilt strains acted as a unit where pronounced spiraling did occur, turning mostly in a clockwise direction except on galactose, where the direction was counter-clockwise. Why this form of growth should have occurred is not clear.

No attempt was made to compare the fungi as to microscopic characters, but an examination of all plates was made to determine the kind and relative abundance of spore types. The wilt fungus is notably (14) a poor sporulator and as a rule little difference was found between strains. Very few macroconidia were observed on any of the media. Microconidia were produced sparingly by 5b and 7b, and to a less extent by 2a on sucrose, maltose, lactose, arabinose, and mannite. Chlamydospores were usually abundant, particularly on pectin. Those media poor in chlamydospore formation—xylose, galatose, and glucose—were also poor in all types of sporulation. Among the other fungi, *Fusarium conglutinans* var. *callistephi* and *F. tracheiphilum* were the most profuse sporulators, both as regards microconidia and macroconidia, exceeding all vascular *Fusaria* studied in that respect.

It appears that the vascular *Fusaria* utilize a wide range of carbohydrate sources, but do least well upon the pentoses, xylose and arabinose. This is in direct contrast to certain cortical *Fusaria* which have a preference for the pentoses (6). All seemed to do uniformly well upon pectin, especially after growth had proceeded far enough to counteract the high acidity of the medium. High concentrations of carbohydrate were used to accentuate color differences (4), and the data are therefore not exactly comparable to similar studies of this sort. However, the set-up given by Fahmy (9) in his work upon *F. vasinfectum*, was duplicated in another experiment, and comparable results were obtained. He found that the best growth of the cotton-wilt fungus occurred on glucose, galactose, sucrose, starch, and lactose in the order named.

DISCUSSION

Variability in monoconidial cultures of the *Fusaria* has been repeatedly observed. The genus *Fusarium* in fact contains some of the most outstanding instances of variation in colony character to be obtained through the use of single-spore methods (12). Brown (5) and recently Leonian (13) found, through the study of sectors from monoconidial parents, that cultural variability is accompanied by a different pathogenesis, usually in the form of a higher or lower virulence. In a survey of some 40 strains of *F. fructigenum* Fries, mostly saltants, Brown reported a wide range of cultural and virulence variability, and noted that the mycelial types were usually the more pathogenic. It was his conclusion that there should be a reduction in the number of species recognized on the basis that many specific names are applied to the variants of a species. Leonian compared the pathogenicities of a large collection of variants obtained from monoconidial cultures of *F. moniliforme* Sheldon. All degrees of virulence were encountered but there appeared to be no rule or order in the behavior of the dissociants, and it proved to be impossible to definitely correlate virulence with colony character.

All evidence from studies upon variation in fungi illustrate the hazard of using single-spore cultures in the study of a species exhibiting variation, unless large numbers of monoconidial cultures are employed. Otherwise only a single component of the species characters may be observed. Accordingly in these studies numbers of monoconidial lines were obtained for each strain, and from all of these types were chosen representatives of the species as obtained. In a portion of the pathogenicity studies the difficulty was obviated by using gross cultures.

The investigations reported here on *Fusarium orthoceras* var. *pisi* have yielded results somewhat comparable to those discussed above. Differences in colony character were at once apparent between original isolations of the fungus from different regions and localities. Differences of the same order between monoconidial lines were found. Moreover, within a given monoconidial line it was possible to assemble, through the phenomenon of dissociation, a group of cultures almost representative of the range in colony type and virulence exhibited by the entire group of strains. Thus a monoconidial parent has been shown in certain instances by its dissociants to possess the potentialities of most of the types of colony character and virulence of the 15 strains studied. It seemed that the careful selection of two or three parents would yield the entire range of strain differences. These variations, although wide, are considered within reasonable species limits. The variability in pathogenesis is always in respect to virulence upon susceptible varieties and bears no absolute correlation to the types of cultural variability. No resistant variety has been found susceptible to any culture, nor has any susceptible variety proven to be resistant to a fungal strain.

The occurrence of variability in the pea-wilt fungus again raises the question of strain origin. In addition to the variation within species, the very close interrelationship of the vascular *Fusaria* has suggested the operation of a sexual stage in nature resulting in the group of vascular parasites, or at least accounting for the variability in the specific groups, as segregants. Not to overlook the possibility that such a combination might be effected in artificial culture, the entire range of strains collected of the wilt fungus were planted side by side in different combinations on agar plates using different media. Other *Fusaria*, including strains of *Gibberella saubinetii* (Mont.) Sacc. and *G. moniliformis* (Sheldon) Wineland were also employed. These trials, although only superficial, gave negative results, but it still does not seem illogical that sexual fusions either occur now in nature producing a diversity of closely allied forms, or did occur, leaving a large assortment of segregants. So nearly, in fact, do some of the vascular *Fusaria* resemble one another, that it would seem that several evolved merely as variants or segregants in respect to pathogenicity. The *Fusaria* concerned in cabbage yellows, aster wilt, and beet wilt, for example, are readily distinguishable only by their parasitic properties, which, however, are sharply defined. These organisms have been termed respectively *F. conglutinans*, *F. conglutinans* var. *callistephi*, and *F. conglutinans* var. *betae*. The pea-wilt fungus, although named *F. orthoceras* var. *pisi* includes within its range strains or variants which so overlap in colony character those of the above fungi as to be practically indistinguishable from them, except in pathogenesis which is again extremely clear cut. The only satisfactory means the pathologist has in differentiating between these four parasites (and possibly certain others) is upon the basis of parasitism, a quality which may logically be recorded in the varietal name of the fungus. In the same way other of the vascular *Fusaria* seem to be evolved from a group type with overlapping characters, and pathogenesis again effects the positive differentiation. There are obviously dangers attending to investing pathogenesis with specific ranking in mycologic nomenclature, but the pathogenic qualities are of great practical value in varietal delimitation. No

cross-inoculation trials between the vascular *Fusaria* and their various hosts have been successful. For the most part they are confined to one host species, and when fungal variations in pathogenicity have occurred, it involved varieties of the host species rather than new hosts.

Lacking knowledge of a sexual phase in the life histories of certain fungi, other explanations have been advanced to account for the variability displayed by the Fungi Imperfecti. Leonian (13) obviated the need of explaining the mechanism of variability by stating that dissociation is a phenomenon whereby a given organism traces the sphere of variability of the species, and it is therefore a highly natural and normal behavior. But even in this light a mechanism must operate in the appearance of the sudden change in a part of a colony where another phase of the species character comes into prominence. Brierley (1, 2) has proposed, while holding that the species in nature is of a collective kind, a mixochimaera theory in explanation of dissociation. Recently Hansen and Smith (10) have presented experimental data in support of this theory which they term "heterocaryosis," based upon nuclear assortment as a result of hyphal anastomosis. That anastomosis is of widespread occurrence among fungi is now an accepted fact, and also that not only unicellular spores but also hyphal cells may be multinucleate. The nuclei of these cells are not presumed to be genetically identical. Upon this basis Hansen and Smith postulate that a hyphal fusion gives rise to a new nuclear complex without nuclear fusion, and that the division of such cells yields units containing different nuclear assortments. It is the assortment of nuclei present in the cells of mycelium that they consider responsible for the thallus character, consequently where hyphal fusions occur in the fungal colony and subsequent cell division gives rise to a different nuclear assortment, a sector or variation originates. The occurrence of a multinucleate condition of unicellular spores accounts for the perpetuation in monoconidial lines of a diverse nuclear content. This theory of heterocaryosis provides a convenient explanation for most variation phenomena observed in the imperfect fungi where the spores contain two or more nuclei. The instances of variation in *F. orthoceras* var. *pisi*, with which this paper deals, might be explained under this theory. In those strains of relative stability, in regard to sectoring, such as 1a, the nuclei of the multinucleate hyphal cells may be considered largely of one kind. Unstable strains on the other hand may have a diverse assortment of nuclei. Thus variants might be expected to appear following anastomosis and nuclear reassortment (10), or as a result of spores being cut off bearing a change in the ratio of nuclear type or number (?), or following mycelial growth from a hyphal cell which did not get its proper quota of kind and number of nuclei. It would seem that convincing proof or disproof of the hypothesis might be obtained through an analytical study of the ascospore cultures of those *Gibberella* species containing marked variation in the *Fusarium* stages. By this means thalli of a unicellular, uninucleate origin would be available for either study of the analysis or synthesis of dissociating colonies.

It appears, however, irrespective of the mode of strain origin, that there is a well-defined constancy in the kind of pathogenicity obtained with all strains of the pea-wilt *Fusarium*, and that the variability of the fungus is expressed in colony character and to some extent in virulence.

SUMMARY

Fifteen strains of *Fusarium orthoceras* var. *pisi*, the fungus causing pea wilt, were collected from eight States and compared in respect to pathogenicity and culture characters.

No evidence was found suggesting that different strains of the wilt fungus vary in host specialization.

Although all strains possessed the same kind of pathogenesis when tested upon a standard group of pea varieties, they varied somewhat in regard to the degree of pathogenesis.

All strains were arbitrarily grouped according to virulence. The differences in virulence were generally not extreme, but were encountered frequently both between monoconidial lines of a strain and between strains.

A comparison of monoconidial cultures representative of the strain types was marked by a wide range of variation in colony character. On the other hand comparison of the pea-wilt fungus with other vascular *Fusaria* showed a striking general similarity and overlapping of characters.

Type strains of the pea-wilt organism exhibited the same general temperature requirements upon artificial media and have a common optimum range for maximum radial growth upon agar plates.

Pronounced differences occurring on the same medium between different strains and between variants from the same single-spored parent thallus were recorded in respect to quality, intensity, rate of pigmentation of the medium, and other characters.

Fusarium orthoceras var. *pisi* and other vascular *Fusaria* studied, showed a wide carbon source preference, growing well on all media tried except the pentoses, xylose and arabinose. Strain differences were brought out to some extent upon these media.

Variation as it occurs in *Fusarium orthoceras* var. *pisi* seems to fluctuate within type limits defined on the one hand by certain general culture characteristics and on the other hand by a rather specific behavior in respect to pathogenicity.

No strain encountered was considered sufficiently divergent in type from the rest to justify the creation of new nomenclature. Indeed, it would rather seem advantageous to simplify the existing nomenclature of the vascular *Fusaria* by a condensation under one species name of those parasites, the culture characters of which so overlap as to make ordinary mycological methods inadequate in their differentiation, distinguishing such parasites by varietal names based upon host pathogenesis.

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INHERITANCE OF REACTION OF WHEAT TO PHYSIOLOGIC FORMS OF *TILLETIA LEVIS* AND *T. TRITICI*¹

By WILLIAM K. SMITH²

Formerly agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry,
United States Department of Agriculture

INTRODUCTION

Recent studies throughout the main wheat-growing regions of the world have demonstrated the existence of numerous physiologic forms within *Tilletia levis* Kuehn and *T. tritici* (Bjerk.) Wint., the two species of fungi causing bunt in wheat.³ The presence of these forms makes the study of inheritance of resistance in wheat crosses somewhat more complicated than previously.

When the situation is considered from the economic point of view, it is apparent that in regions in which numerous forms are present the breeding of varieties resistant to bunt and desirable in other respects can now proceed with more assurance of success. Consideration, however, should be given to the fact that hybridization between physiologic forms and the possible production of new forms have been demonstrated (11).⁴ In the Pacific Northwest only one form of *Tilletia tritici* was known to be present prior to 1918. Recent surveys (18) have shown that physiologic forms of *T. levis* and new forms of *T. tritici* are now widespread throughout the wheat-growing portions of this area. Heald and George (17) have shown that seed treatment with various fungicides, effective elsewhere, is of limited value for winter wheat in this area. Moreover, some varieties that are resistant when inoculated with the smut prevalent a decade ago are very susceptible to some of the recent forms. A promising method of coping with this condition lies in the production of new varieties resistant to these fungi and possessing other desirable qualities. Progress can be made more rapidly in this undertaking when the mode of inheritance of reaction to bunt in various varietal crosses is known.

PREVIOUS WORK

Earlier studies on the genetics of reaction to bunt have been reviewed by Briggs (2) and need not be considered here in detail; only the more pertinent features will be mentioned.

In all the investigations reported, the rough-spored species, *Tilletia tritici*, has been used as the inoculum. Gaines (12, 13) and Gaines

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² The writer is indebted to Dr. F. D. Heald, head of the Department of Plant Pathology, State College of Washington, for suggestions regarding the manuscript; to Dr. E. F. Gaines, professor of genetics, State College of Washington, for suggestions and criticisms in the course of the study; and to members of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, for suggestions arising from a critical reading of the manuscript.

³ A third species of *Tilletia* infecting wheat was discovered recently in India (21).

⁴ Reference is made by number (italic) to Literature Cited, p. 104.

and Singleton (14) found that in the inheritance of reaction to bunt in the crosses studied many factors were concerned, the number of which could not be determined except in a cross between Marquis and Turkey in which a 2-factor difference was indicated. In some crosses, resistance was inherited as a recessive. In two crosses, well-defined transgressive segregation occurred, some of the progenies being more resistant and others more susceptible than the parents.

Briggs (2) studied the inheritance of reaction to bunt in hybrids between three susceptible wheats and two wheats (Martin and Hussar) which are resistant to the physiologic form of bunt used in the investigations at the California Agricultural Experiment Station. It was shown that resistance behaves as a dominant in inheritance in these crosses. A single main factor was found to govern the resistance of Martin, whereas Hussar was found to differ from the susceptible wheats by at least two dominant factors, one of which is the same as the factor in Martin. The expression of a second factor is described in a recent publication (4). White Odessa, another variety resistant to the form of bunt used in the experiments of Briggs, has been found (5) to differ from susceptible wheats by one main dominant factor. Briggs (6) has also shown that Banner Berkeley carries the same factor for resistance as Martin.

In Germany a number of crosses between spring and winter wheats were investigated for reaction to bunt by Knorr (19). His results indicated that resistance was inherited as a recessive; the number of factors involved could not be determined. In crosses between winter wheats, Giesecke (15) concluded that resistance was recessive and that multiple factors were responsible for the results obtained. Transgressive segregation was found in a cross between two moderately susceptible wheats.

Because the presence of physiologic forms in the two species of fungi causing bunt was discovered only recently, no studies on the reaction of progenies of a cross to different forms have as yet been described; nor have investigations been reported on the inheritance of reaction to *Tilletia levis*.⁵ Consideration was given to these phases in the investigation reported herein.

MATERIALS

Hope (*Triticum vulgare*⁶ Vill.) (C. I. 8178)⁷, a spring wheat, was one of the parents in all crosses tested in this study. It is described by Clark and Ausemus (7), and McFadden (20) discusses its agronomic qualities. Of the other parents, Jenkin (*T. compactum* Host) (C. I. 5177) was the only spring wheat; it is described by Clark, Martin, and Ball (9). White Odessa (C. I. 4651) and Ridit (C. I. 6703), varieties of *T. vulgare*, were the winter-wheat parents; both are described by Tisdale et al. (24), and additional data on Ridit are presented by Clark, Love, and Parker (8).

Three so-called physiologic forms of *Tilletia tritici* (T-1, T-2, T-3) and two forms of *T. levis* (L-4, L-5) were used in the tests. These forms were isolated from collections of bunt made in various parts of

⁵ Since this manuscript was completed, the reactions of two crosses to two collections of *Tilletia levis* and two of *T. tritici* have been described (1).

⁶ According to the rules of botanical nomenclature the name of this species is *Tritium aestivum*, but as *T. vulgare* is in general use among agronomists, the writer gives preference to that form.

⁷ Accession number of the Division of Cereal Crops and Diseases.

the Pacific Northwest and were obtained by culturing on differential hosts. The reaction of some of the parental varieties to these forms can be shown in the results of a rod-row test made in 1929, the results of which are in close agreement with those of a similar test made in 1930. In both seasons, environmental conditions were very favorable for infection, as evidenced by high percentages of bunt on the susceptible varieties. The results of the test made in 1929 are presented in table 1.

TABLE 1.—Reaction of varieties of wheat to three forms of *Tilletia tritici* and two forms of *T. levis*, 1929

Variety	C I No.	Percentage of wheat attacked by indicated form of bunt				
		T tritici			T levis	
		T-1	T 2	T 3	L-4	L-5
Hybrid 128	4512	68	83	90	84	90
Turkey	5175	6	86	2	71	72
Albit	8275	0	75	77	44	0
Martin	4463	0	19	71	51	0
Hussar	4843	0	25	0	39	0
Ridit	6703	0	2	0	6	3
Hope (fall sown)	8178	51	60	42	53	78
Hope (spring sown)	8178	0	0	0	0	0
Jenkin (spring sown)	5177	92	78	73	58	56

In table 1 the five physiologic forms of *Tilletia* are clearly differentiated on the two winter wheats, Martin and Hussar. T-2 caused 25 percent of bunt on Hussar and 19 percent on Martin, whereas these wheats were smut free when inoculated with T-1. Martin distinguishes T-1 from T-3. The 25 percent on Hussar with T-2 is in sharp contrast to zero with T-3.

Martin, however, is equally effective as a differential between T-2 and T-3. Bunt developing on Martin (23) after inoculation with T-2 is very different from that caused by any other form of the fungus under test. T-2 gives rise to smut balls which at maturity are only slightly larger than normal ovaries before fertilization; of the spores which they contain, some appear mature, but a large proportion have unthickened or partly thickened walls and seem to be in various stages of development. Because of the partly developed smut balls, heads of Martin infected with T-2 resemble sterile heads in external appearance, and can readily be distinguished from heads of this variety infected with any of the other four physiologic forms. Albit, Martin, and Hussar serve to differentiate the two forms of *Tilletia levis*.

A peculiar condition has been observed in Hope. Prior to the growing season of 1929, Hope had been highly resistant to bunt in tests at Pullman, Wash. The winter of 1928-29 was mild, and seeds of this variety inoculated with different collections of bunt and sown in the fall of 1928 produced plants which lived through the winter and exhibited at maturity from 42 to 78 percent of bunt. Later trials have confirmed the conclusion that Hope, when sown at the normal seeding time in spring, is resistant to all forms of bunt with which it has been tested, but when sown in the fall is susceptible. This condition is described in greater detail in another paper (22).

METHODS

The parental material and hybrids were grown in the nursery field which forms a part of the farm of the State College of Washington. The field is divided into three parts, and on these a 3-year rotation (sweetclover, summer-fallow, cereal nursery) is maintained. The summer-fallow is kept free from plant growth, and thus the possibility of the appearance of volunteer grain in the experiments is reduced to a minimum. F_1 and F_2 hybrids were protected as much as possible from bunt infection by treatment with copper carbonate.

The necessity of maintaining the physiologic forms of the fungi free from contamination is obvious. Judicious selection of a host variety for each form has been of much service. For example, from the results presented in table 1, if inoculum of T-2 were collected on Hussar neither T-1 nor T-3 would be present. Similarly, if T-3 were collected on Martin, neither T-1 nor T-2 would be present, provided the abnormal smut heads from an infection of this variety by T-2 were not overlooked. It is recognized that the five forms mentioned above may not be pure; some of them may be composites of two or more forms or may be heterozygous for pathogenicity. However, in testing these forms on various hosts and using inoculum from different varieties in two growing seasons, there has been no indication of mixture.

Inoculum of each physiologic form was obtained from selected varieties as soon as the crop had matured. Hybrid seeds were counted out and arranged for inoculation along with the necessary checks. Inoculation was made by placing a quantity of powdered smut in a glass vial with each lot of seeds and shaking vigorously until the seeds appeared well blackened. The excess smut not adhering to the seeds was then sifted out. During these operations every precaution was taken to prevent the contamination of any form by spores from any other.

Sowing was done in either rod rows or $\frac{1}{2}$ -rod rows, the seeds being sown approximately $2\frac{1}{2}$ inches apart to facilitate the separation of single plants at harvest. Care was taken that all rows and different parts of each row should be, as far as possible, of the same depth, approximately 3 inches. The seed for each experiment was sown on the same day, except in one instance when sowing extended over two consecutive days.

It is recognized that in certain parts of the Pacific Northwest at the time of fall seeding the surface soil may be heavily contaminated with wind-blown spores of *Tilletia*. By means of spore traps, Heald and George (17) in 1916 demonstrated a fall of over 13,000 spores per square inch in a period of seven days when the dissemination of spores was at its height. Sterilization of the surface soil in the field is not practicable and may be undesirable since it may upset the biological balance in the soil. In spite of this possible source of contamination, no difficulty has been experienced in keeping the forms of bunt pure; and there has been ample opportunity in these tests for admixtures to become apparent. For example, considering some of the varieties listed in table 1, in the series inoculated with T-1, any admixture of T-2, T-3, or L-4 would smut Albit and Martin; likewise, admixture of T-2 or L-4 would smut Hussar. Small percentages of bunt would also be apparent on certain varieties in some of the other four series and in other wheats not listed in table 1. No such infections have been found in tests conducted over a period of two years. The absence of contamination may be accounted for

by the fact that (1) wind-blown spores were almost entirely of T-1, which does not smut the differential wheats Albit, Martin, and Hussar, and (2) all seeds, when planted, carried the maximum spore load, which may be in the neighborhood of 150,000 spores per kernel (16).

At harvest the data on bunt were recorded by grouping the plants according to the proportion of smutty heads. In studies on the inheritance of reaction to bunt, different methods of determining the reaction of parents and progenies have been employed. Gaines (13) separated the plants of each row into three piles—bunt free, wholly bunted, and partly bunted—and recorded the number of plants in each. The total percentage of bunt in each row was computed by the formula $a \times b + c = d$, in which, in any row:

a = percentage of bunted heads in the partly bunted plants,

b = percentage of partly bunted plants,

c = percentage of wholly bunted plants, and

d = total percentage of bunt.

Briggs (2) maintained that in a study of inheritance the plant should be considered as the unit. He separated the plants in each row into two piles, bunt free and bunted. The total number of plants and the number of bunted plants were recorded and the percentage of bunt calculated. A plant was classed as bunted if it showed a trace of bunt. Both Giesecke (15) and Knorr (19) have employed this method in inheritance studies.

In comparing the bunt reaction of different varieties of wheat it seems that, while two varieties may have the same proportion of plants bunt free, one variety may have consistently a large proportion of plants with a trace of bunt, while the other may have a large proportion of the plants wholly bunted. If, under such conditions, all plants showing any degree of smutting are placed in one group, genetic differences in reaction may be concealed. This point is illustrated in table 2.

TABLE 2.—Distribution of plants among the five bunt-percentage groups and percentage of bunt in each row of duplicate rows of Hybrid 128 and Martin wheat inoculated with physiologic form T-2 and grown in 1930

Variety	Row No	Number of plants in bunt-percentage group indicated					Total number of plants	Percentage of bunt in row
		0	20	50	80	100		
Hybrid 128	1	5	0	1	2	20	28	79
	291	3	1	0	3	26	33	87
Martin	5	3	7	5	6	0	21	41
	299	4	12	8	4	1	29	37

$$\text{Percentage of bunt in row 299} = \frac{(20 \times 12) + (50 \times 8) + (80 \times 4) + (100 \times 1)}{29} = 37$$

In the present study the plants in a row were first pulled, then sorted into five bunt-percentage groups as follows:

0 percent = all heads bunt-free.

20 percent = up to one-third of heads bunted.

50 percent = one-third to two-thirds of heads bunted.

80 percent = above two-thirds, but not all, of heads bunted.

100 percent = all heads bunted.

A spike which contained both kernels and smut balls was considered smutty. The number of plants in each of the five groups was then

counted and recorded. Computation of the percentage of bunt is illustrated in row 299 of table 2; calculation was made to the first decimal place in percentages less than 10 and to the nearest whole number in those greater than 10.

It is apparent from table 2 that the number of bunt-free plants in the two varieties, Hybrid 128 and Martin, is approximately the same, whereas the distribution among the bunt-percentage groups is strikingly different. The results with these varieties in 1929 corresponded closely to those obtained in 1930. It is apparent that if, in this example, percentage of bunt is based on two groups, bunt-free and bunted, the percentage of bunt in the two varieties is approximately the same; with reference to the degree of smutting, however (table 2), the percentage of bunt in one variety is approximately double that in the other. Differences of a similar nature, though usually not so marked, have been observed in other varieties. It would be conceded that these differences between varieties are due to differences in reaction. Similar differences would be expected between hybrids. It may be advantageous, therefore, in the genetical study of reaction to smut, to record the data in such a manner as to show the plant distribution with respect to degree of smutting in each row as well as the total percentage of smut.

EXPERIMENTAL RESULTS

REACTION OF HOPE $\times$ JENKIN TO PHYSIOLOGIC FORMS OF BUNT

Crosses were made between Hope and Jenkin in 1927, and the first generation was grown in 1928. In the spring of 1929 the seeds obtained from the F_1 plants were spaced 4 to 5 inches apart in rows 18 inches apart to allow for the vigorous development of each plant; each F_2 plant consequently gave a good yield of seed. Hope, when sown in spring, has been resistant to all the physiologic forms of bunt used in the tests at Pullman, whereas Jenkin has been susceptible to all forms. One of the objects of the present study was to determine whether the factors responsible for resistance to any one form were the same as those for resistance to any other form. Other phases under investigation were (1) the number of factors concerned in the inheritance of reaction to various bunt forms in this cross, and (2) the relationship, if any, between reaction to bunt and such characters as glume color and length of awn. Accordingly, from each of 195 F_2 plants, three sets of 75 seeds were counted out. In each of the three series a pair of check rows, one of each parent, was included after every tenth hybrid row. The first series was inoculated with T-3, the second with L-5, and the third with a mixture of T-1, T-2, and L-4. Data were recorded in the manner described, and the distribution of progenies and parents, according to their reaction to the forms of *Tilletia*, is shown in table 3 and in figure 1.

In the study of the reaction of wheat varieties and hybrids to bunt it has been observed (14, p. 170) that a given percentage difference between two resistant types is much more significant than the same percentage difference between two susceptible types; that, for example, a segregate or variety

which produces an average of 1 percent of bunt is evidently quite different from a variety which produces 10 percent, but a variety which produces 70 percent is not so different from one which produces 80 percent.

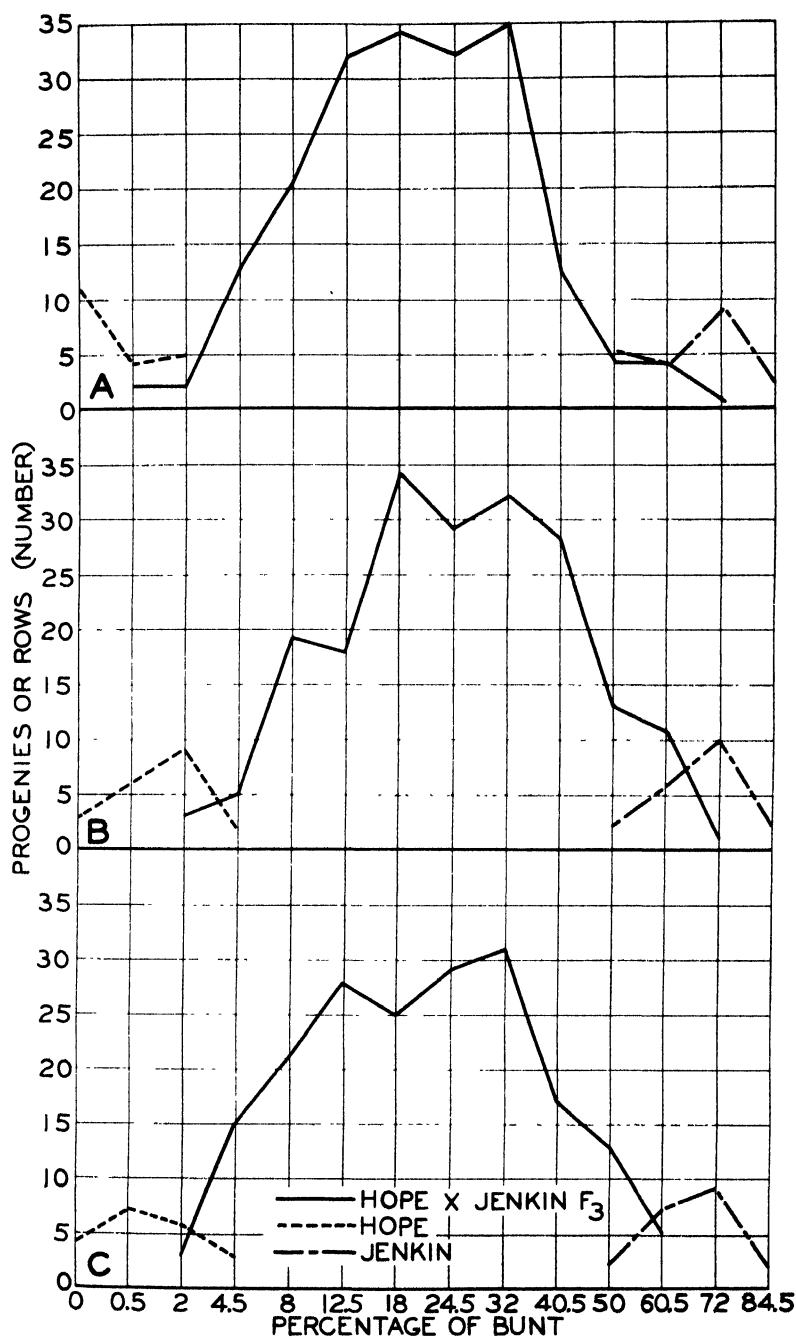


FIGURE 1.—Distribution among bunt-percentage classes of parental rows and F_3 progenies of Hope $\times$ Jenkin in three series inoculated with different physiologic forms and sown April 6, 1930: A, Series 1, inoculated with T-3; B, series 2, inoculated with L-5; C, series 3, inoculated with a mixture of T-1, T-2, and L-4.

TABLE 3.—Percentage of bunt on parents and F_3 progenies of Hope $\times$ Jenkin when inoculated with different physiologic forms and sown on April 6, 1930

Parent or F ₁	Series No	Inoculated with form -	Number of rows in the bunt-percentage class indicated																Total number of rows
			0	0.5	2	4	5	8	12	15	18	24	32	40	50	60	72	84	
Hope	1	T-3	11	4	5	—	—	—	—	—	—	—	—	—	—	—	—	—	20
Jenkin			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	20
Hope × Jenkin			—	2	2	13	21	32	34	32	35	12	4	4	1	—	—	—	192
Hope	2	L-5	3	6	9	2	—	—	—	—	—	—	—	—	—	—	—	—	20
Jenkin			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	20
Hope × Jenkin			—	—	3	5	19	18	34	29	32	28	13	11	1	—	—	—	193
Hope	3	T-1, T-2, and L-4 (mixture)	4	7	6	3	—	—	—	—	—	—	—	—	—	—	—	—	20
Jenkin			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	20
Hope × Jenkin			—	—	3	15	21	28	25	29	31	17	13	5	—	—	—	—	187

In the classification of Gaines and Singleton the differences between the class centers, with the exception of the difference between the first two classes, constitute an arithmetical progression in which the values increase by 2.5. Other investigators of the inheritance of reaction of cereals to the various smuts have grouped hybrid and parental rows in classes of 5 or 10 percent of smut. It may be desirable, however, in a study of the distribution of hybrid progenies and parents in reaction to smut, to arrange the progenies and parental rows in classes which are of approximately equal value in resistance or susceptibility.

In the present study (table 3), the first class contains rows showing no smut, while for the other classes the class intervals form an arithmetical progression in which the interval of the first is one and that of the others increases by one. Such a classification gives some 14 or 15 classes; if the number of classes is less than these, tendencies in distribution may not become apparent. Perhaps the best test of the validity of any such method is an examination of the distribution of the check rows. Since the parental rows were distributed in pairs—one row of Hope and one of Jenkin—throughout the nursery, the resistant parent was subjected to approximately the same environmental conditions and should show the same fluctuations as the susceptible parent. The data in table 3 show that Jenkin fluctuated among four classes in each of the three series. A similar situation was found in Hope. The reactions ranged through 4 classes in series 2 and 3 and probably would have ranged through 4 in series 1 if the degrees of resistance in the 11 rows showing no bunt had been given. Moreover, examination of the reaction of parental rows in table 7 indicates that the distribution through reaction classes of types intermediate between highly susceptible and highly resistant, e.g., White Odessa, is similar to that found in the extremes as represented by Hope and Jenkin in table 3.

Figure 1 shows clearly the high resistance of Hope and the high susceptibility of Jenkin in each of the three series. Because of the sharply contrasting reactions of the two parents, the nature of the segregation of bunt reaction in the F_3 progenies can be more readily determined.

Dominance of either resistance or susceptibility has frequently been ascertained from a comparison of the reaction of the F_1 hybrids with the parents. However, in a segregation involving a number of factor pairs, the expression in the F_1 plants may be the result of the dominance of resistance in certain factor pairs and the dominance of sus-

ceptibility in others. Only by a study of each factor pair can dominance be accurately determined. This has not been attempted in the Hope $\times$ Jenkin cross. However, a comparison of the reaction of the F_3 progenies with that of the parents (fig. 1) gives no indication of the dominance of either resistance or susceptibility.

There is a striking resemblance between the distribution of progenies in each of the three series, a condition which suggests the interaction of the same number of factors in each series. Before investigating the possible number of factors involved, the three series may be compared in more detail.

It has been mentioned that, of each hybrid progeny, one part was inoculated with T-3, a second part with L-5, and a third with a mixture of T-1, T-2, and L-4. The percentages of bunt in these portions of each progeny have been correlated, and the results are shown in table 4.

TABLE 4.—Correlations between series containing portions of F_3 progenies of Hope $\times$ Jenkin, inoculated with different physiologic forms of bunt, series 1 with T-3, series 2 with L-5, and series 3 with a mixture of T-1, T-2, and L-4

Series correlated	Number of progenies in the comparison	Coefficient of correlation
1 with 2	190	0.69 $\pm$ 0.03
1 with 3	184	.69 $\pm$.03
2 with 3	186	.66 $\pm$.03

The data in table 4 show that in each of the three comparisons there is a significant and high degree of correlation between the percentage of bunt produced in the several portions of each progeny. The value and similarity of the coefficients of correlation indicate that at least some of the factors responsible for resistance to one form are the same as those responsible for resistance to the other forms.

The relationship between the series may be determined more exactly by examination of the correlation tables. Since the three tables are similar, only one will be presented. The correlation between series 1 and series 3 appears as table 5.

TABLE 5.—Correlation between the percentages of bunt in two portions of each of 184 F_3 progenies of Hope $\times$ Jenkin, one portion inoculated with physiologic form T-3 (series 1), the other with a mixture of physiologic forms T-1, T-2, and L-4 (series 3)

Bunt-percentage class in series 1	Number of plants in indicated bunt-percentage class in series 3														Total
	2	4	5	8	12	5	18	24	5	32	40	5	50	60	
0 5	1			1											2
2				1	1										2
4 5	1		5	3	3					1					13
8			4	9	3		2	2							20
12 5			3	2	8		6	2	5		1				28
18	1		1	3	9		5	5	6			2			33
24 5					1	1	4	10	8	5	1				30
32						1	3	6	6	9	6	3		1	35
40 5								2	2	2	2	3		1	12
50									2				2		4
60 5											1	1		2	4
72														1	1
Total	3	13	21	28	25	29	31	17	12	5					184

In the distribution of data in table 5 for the 12.5 percent class of series 1, the array of series 3 extends through 8 classes; similarly, in the 18 percent class and 32 percent class of series 1, the array of series 3 is distributed through 8 classes, whereas the arrays of the other series extend through only 7 classes or less. The percentage of bunt in the Jenkin check rows of each of the three series (table 3) fluctuates among 4 classes; a similar variation exists in the Hope check rows. If, therefore, the percentages of bunt in the Jenkin check rows of series 1, for example, were correlated with corresponding Jenkin rows in series 3, in no case would one class of one series have an array of more than four classes of the other series. All rows of Jenkin, however, have the same genotypic constitution. The condition in the hybrids is somewhat different. In any class of each series there is a variety of genotypes. Assuming that the fluctuations in percentage of bunt in the hybrids are the same as those in the parents, the genotypes in any one class of the hybrids could theoretically give fluctuations among three classes on either side of that class, giving in all a range of seven classes. In table 5 variations through eight classes, instead of seven, occur, but this difference is probably not significant. If, therefore, a correlation table were made from two series that had been inoculated with the same physiologic form of bunt instead of with different forms as in table 5, the expected distribution, on the basis of the fluctuations found in the parental rows, would not differ materially from that presented in table 5. It may therefore be concluded that the factors in Hope responsible for resistance to the bunt form used as inoculum in series 1 (T-3) are the same as those responsible for resistance to the forms used in series 3 (T-1, T-2, and L-4). Since a similar situation is found in the correlation tables of series 1 with 2 and series 2 with 3, the conclusion may be extended to include resistance to the form of smut with which series 2 (L-5) was inoculated. The similarity of the distribution of hybrid rows in the three series in figure 1 adds weight to this conclusion.

One striking feature in a factorial analysis of the data (table 3) is the relatively small number of resistant progenies and the entire absence of bunt-free progenies; of equal significance is the small number of progenies as susceptible as the Jenkin parent. These features, together with the great preponderance of progenies having reactions intermediate between those of the two parents, indicate the presence of a number of factors. Because of the importance of extremes of susceptibility and resistance in the genetic analysis of the data, the plant distribution in some of the most resistant progeny rows, together with that of the nearest check rows of Hope, is given in table 6.

Evidence has been presented which indicates that in this cross the factors for resistance against the invasion of the plant by any one of the physiologic forms of bunt tested are the same as those for resistance against any other form used in this study. Therefore, any progeny that has the same genotype as Hope should not have a higher percentage of bunt in any of the three series than would be possible in Hope under the conditions of this experiment. The probable error in terms of percentage of bunt can not be used to advantage in determining the potential range of the parents under these conditions because of the fact that in a study of this nature a given range in percentage of bunt, e.g., 5 percent, is of smaller significance at the sus-

ceptible end of the curve than at the resistant end. Approximate limits, however, may be defined from a study of the distribution of the parental rows through the bunt-reaction classes. Such a determination may be made from the data on the parents in table 3. Moreover, it may be added that the highest percentage of bunt in Hope in series 1 is 2.4; in series 2, 3.0; and in series 3, 4.2. From table 6 it appears that on this basis only progenies 860-35 and 861-16 come within the probable limits of Hope in all three series. However, Briggs (3) has shown that modifying factors may operate in the reaction to bunt of certain crosses. Allowing for the operation of such factors in addition to the main factors in the Hope $\times$ Jenkin hybrids, progenies 861-62 and 861-66 might also be considered to have the same main factors as Hope, giving in all from a total of 195 progenies 4 as resistant as the resistant parent. The other 3 progenies listed in table 6 show significantly large deviations from the reaction of Hope.

TABLE 6.--Distribution in bunt-percentage groups of plants in the seven most resistant F_3 progenies of Hope $\times$ Jenkin compared with that in the nearest check rows of Hope and with that of all check rows of Hope and Jenkin, in each of the three series

Progeny no. or variety	Series No	Number of plants in the bunt-percentage group indicated					Percentage of bunt in row
		0	20	50	80	100	
860-35	1	46	2	0	0	0	0.8
Hope	1	36	0	0	0	0	0
860-35	2	42	2	1	0	0	2.0
Hope	2	44	5	1	0	0	3.0
860-35	3	39	4	0	0	0	1.9
Hope	3	30	3	1	0	0	3.2
861-16	1	42	6	1	0	0	3.5
Hope	1	28	1	0	0	0	.7
861-16	2	45	9	0	0	0	3.3
Hope	2	43	1	0	0	0	.5
861-16	3	47	2	2	0	0	2.7
Hope	3	39	3	0	0	0	1.4
861-62	1	52	4	2	0	0	3.1
Hope	1	35	2	1	0	0	2.4
861-62	2	39	15	0	1	0	6.9
Hope	2	37	1	0	0	0	.5
861-62	3	38	10	2	0	0	6.0
Hope	3	39	5	0	0	0	2.3
861-66	1	41	4	0	0	0	1.8
Hope	1	35	0	1	0	0	1.4
861-66	2	50	5	1	0	0	2.7
Hope	2	46	4	0	0	0	1.6
861-66	3	30	17	0	0	0	7.2
Hope	3	43	7	0	0	0	2.8
861-69	1	40	4	1	0	0	2.9
Hope	1	35	0	1	0	0	1.4
861-69	2	26	15	3	2	1	15.1
Hope	2	46	4	0	0	0	1.6
861-69	3	27	9	3	2	0	12.0
Hope	3	43	7	0	0	0	2.8
861-81	1	31	9	2	1	0	8.4
Hope	1	44	0	0	0	0	0
861-81	2	31	5	0	0	0	2.8
Hope	2	39	2	0	0	0	1.0
861-81	3	43	3	2	1	0	4.9
Hope	3	34	9	0	0	0	4.2
861-109	1	48	2	0	0	0	.8
Hope	1	42	0	0	0	0	0
861-109	2	48	10	0	0	0	3.4
Hope	2	31	2	0	0	0	1.2
861-109	3	41	10	3	3	0	10.4
Hope	3	38	5	0	0	0	2.3
Hope (all checks)	1	725	9	4	0	0	1.4
	2	701	41	5	1	0	1.4
	3	825	52	2	1	0	1.4
Jenkin (all checks)	1	193	54	85	229	333	63.7
	2	183	48	68	200	425	68.0
	3	210	61	51	189	427	65.7

In a similar manner a comparison was made between the reactions of the most susceptible progenies and those of the susceptible parent. Consideration was given, as in the resistant progenies, to the percentage of bunt in each row, but of much significance is the distribution of the plants of each row in the five bunt-percentage groups. The typical distribution for Jenkin in the three series is given in table 6. Allowing for the presence of modifying factors, only four progenies were considered to have the same main factors for reaction to bunt as Jenkin.

In view of these conclusions concerning the 4 resistant and 4 susceptible progenies and the general distribution of the 195 progenies with respect to reaction to bunt in the three series, it is probable that under the conditions of this experiment Hope and Jenkin differ by three main factors in reaction to the five physiologic forms of bunt.

REACTION OF HOPE $\times$ WHITE ODESSA AND OF HOPE $\times$ RIDIT TO FORM L-4

Crosses were made in 1926 between Hope and each of the two winter wheats, White Odessa and Ridit. F_1 plants were grown in 1927. F_2 plants were grown in 1928 from seeds treated with copper carbonate to reduce bunt infection to a minimum. From the seed produced by each F_2 plant two sets of 38 seeds each were counted out. One series was sown in the fall of 1928 and the other in the spring of 1929, each lot occupying a half-rod row; both series were inoculated with L-4. After every 20 rows of hybrids a row of each parent was sown. In the spring-sown material scarcely a trace of bunt appeared, although susceptible wheats inoculated with the same physiologic form and sown at the same time showed up to 56 percent of bunt. Apparently the winter wheats carry at least some of the same factors as Hope for resistance to L-4 in spring seeding. The data on the amount of bunt in these crosses in the fall-sown series are presented in table 7 and are shown graphically in figure 2.

TABLE 7.—Distribution in bunt-percentage classes of parents and F_2 progenies of Hope $\times$ White Odessa and Hope $\times$ Ridit, inoculated with physiologic form L-4 and sown October 13, 1928

Parent or F ₂	Number of rows in bunt-percentage class indicated														Total number of rows
	0	0.5	2	4.5	8	12.5	18	24.5	32	40.5	50	60.5	72		
Hope.....							1	2	1	1	2	2		9	
White Odessa.....				2	4	4	1	1						12	
Hope × White Odessa.....	1		2	4	10	17	21	48	32	32	34	18	5	224	
Hope.....								2	5		1	2		10	
Ridit.....	6	2	3	1										12	
Hope × Ridit.....	37	15	20	8	28	21	14	22	18	12	14	4	2	215	

In summarizing the reactions of the progenies of the two crosses in table 7, only progenies with 15 or more plants were included. It would have been desirable to eliminate also parental rows having less than 15 plants. However, winterkilling and poor germination considerably reduced the number of plants in the rows of the Hope parent; rows having 11 or more plants were therefore included in table 7. Even on this basis the number of rows of Hope is less than that of the other parent in both crosses. All rows of White Odessa and Ridit contained more than 15 plants.

It will be noted in table 7 that the reactions of the parent varieties to form L-4 fluctuate among 4 to 6 classes, whereas in table 3 the parental reactions range through only 4 classes. However, as was pointed out previously, the Hope $\times$ White Odessa and Hope $\times$ Redit

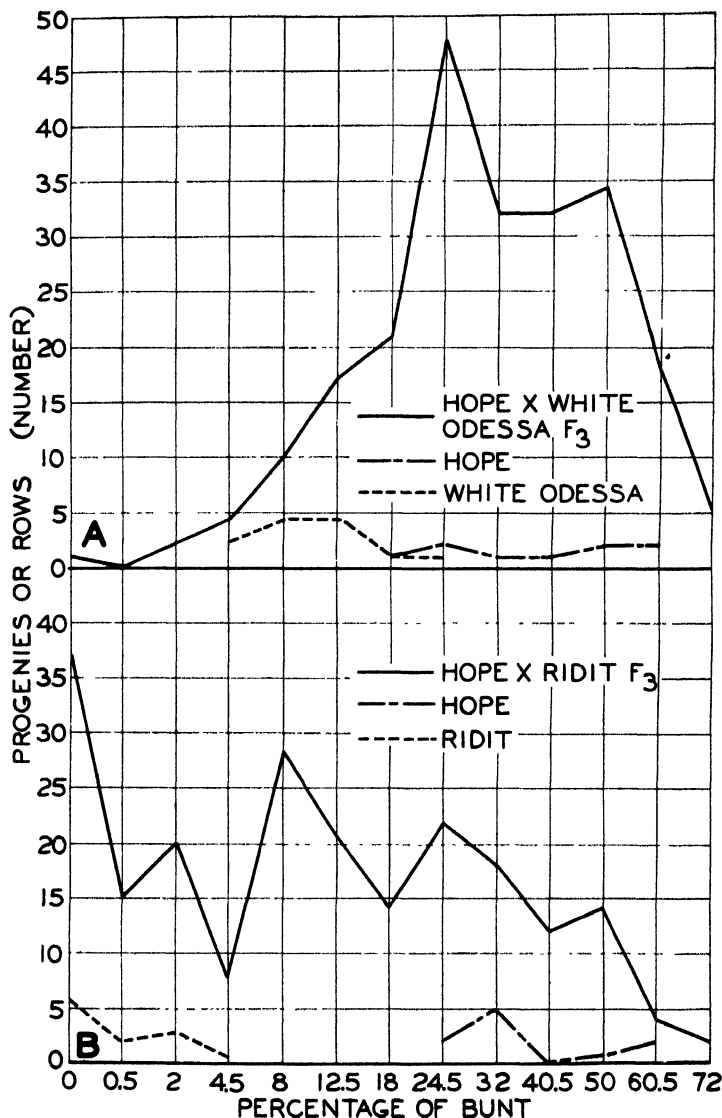


FIGURE 2.—Distribution among bunt-percentage classes of parental rows and F_3 progenies of (A) Hope $\times$ White Odessa and (B) Hope $\times$ Redit inoculated with physiologic form L-4 and sown October 13, 1928.

populations were tested in $\frac{1}{2}$ -rod rows while the Hope $\times$ Jenkin cross was tested in rod rows. It is apparent that the rod-row test is much superior to that made in the $\frac{1}{2}$ -rod row for the analysis of the genetic factors for reaction to bunt.

The number of factors concerned in the Hope $\times$ White Odessa progenies can not be determined from this test, because the reactions of the parental rows overlap and show considerable variability owing to the small number of plants per row. One progeny consisting of 32 plants was free from bunt. Seeds from the same F_2 plant were inoculated with L-4 and sown along with the parents and other progenies in the fall of 1929. Although this progeny was not immune in the retest, it was more resistant than the White Odessa parent. There is a strong indication that much natural crossing occurs at Pullman in certain seasons, and it is possible that this progeny is the result of a natural cross on one of the F_1 plants. It may also be the result of an admixture. Such an interpretation is strengthened by the fact that the general distribution of the progenies (fig. 2, A) does not indicate that the reaction of this progeny is the result of transgressive segregation.

In the Hope $\times$ Redit cross a different situation exists. The high resistance of Redit is sharply contrasted with the moderate susceptibility of Hope. A single-factor difference for reaction to this form is suggested from the distribution of the progenies. A more accurate interpretation, however, can be made from examination of the degree of smutting of plants within each progeny and each parental row. The reactions of the parents planted at intervals among the hybrid progenies may be compared in the summary presented as table 8.

TABLE 8.—Percentage of plants in the five bunt-percentage groups in the parental rows as distributed among the Hope $\times$ White Odessa and Hope $\times$ Redit F_3 populations

Variety	Percentage of plants in bunt-percentage group indicated					Number of—	
	0	20	50	80	100	Plants	Rows
Hope	45	8	14	22	11	279	19
White Odessa	73	14	10	2	1	346	12
Redit	97	3	.6	0	0	327	12

The data of table 8 show that of 327 plants in the Redit rows only 3 per cent were in the 20 per cent bunt group and only 0.6 per cent in the 50 per cent group. Progenies having the resistance of Redit therefore would not have any wholly bunted plants. Furthermore, 29 F_3 progenies of Hope $\times$ Redit, the reaction of which could be classified with difficulty, were retested in 1930 with the same form of bunt. Using these data in connection with the percentages of bunt in the rows of the progenies and the parents, the hybrids were classified, and the resulting data were tested for goodness of fit (10) to a 1:2:1 ratio in table 9. A value of 0.16 for P indicates a reasonably good fit.

TABLE 9. —Test for goodness of fit to a 1:2:1 ratio of 215 F_3 progenies of Hope $\times$ Redit in reaction to bunt form L-4

Item	Goodness of fit for indicated progenies			Total
	Resistant	Heterozygous	Susceptible	
Observed (m + v)	66	100	49	215
Calculated (m)	53.75	107.5	53.75	215
$\frac{\chi^2}{m}$	2.792	.523	.420	3.735

$P=0.16$.

Mention already has been made of the difficulty in determining accurately the presence or absence of dominance. In the Hope $\times$ White Odessa cross the number of factor pairs involved in reaction to form L-4 can not be ascertained. However, the dominance or partial dominance of susceptibility is suggested from the distribution of the F_3 progenies (fig. 2, A).

In the Hope $\times$ Redit cross a single-factor difference is indicated. The presence or absence of dominance could be readily ascertained from a comparison of the reaction of F_1 plants with that of the parents, but seeds for this test were not available. Nor can an accurate determination be made from the F_3 progenies because of the high variability in the parental rows resulting from the small number of plants per row. However, the distribution of F_3 progenies in Figure 2, B, suggests the absence of dominance of either resistance or susceptibility.

RELATIONSHIP OF BUNT REACTION TO OTHER CHARACTERS

In the F_3 progenies of all three crosses, data were obtained on length of awn, color of glume, and winter or spring habit of growth, wherever these characters differentiate the parents. An attempt was made to discover, by means of the correlation-ratio method, any relationship in inheritance between bunt reaction and these three characters. In no instance was there more than a suggestion of any such association.

SUMMARY

Hope, a spring variety of wheat (*Triticum vulgare*) which, when sown in spring, is highly resistant to three physiologic forms of *Tilletia tritici* (T 1, T-2, T-3) and two forms of *T. levis* (L-4, L-5) but which, when sown in the fall, is susceptible to the five forms, was crossed with Jenkin, a spring variety of wheat (*Triticum compactum*) which is susceptible to the five forms.

The seed of each of 195 F_2 plants was divided into three parts; one part was inoculated with T-3, another with L-5, and the third with a mixture of the remaining three forms, T-1, T 2, and L-4.

The factors in Hope for resistance to any one of these five physiologic forms seem to be the same as those for resistance to any of the others.

The results can be explained satisfactorily on the basis of three main factors for resistance, with no indication of the dominance of either resistance or susceptibility.

Crosses were made between Hope and the winter wheats, White Odessa and Redit. F_3 progenies were tested with L-4, to which White Odessa is moderately resistant and Redit highly resistant; one portion of each progeny was sown in the fall, another portion in the spring.

The spring-sown series showed scarcely a trace of bunt, indicating that the winter wheats, White Odessa and Redit, carry at least some of the same factors as Hope for resistance to L-4 in spring sowing.

In the fall-sown series, the number of factors for reaction to L-4 in the Hope $\times$ White Odessa cross could not be determined. Apparently the reaction of Hope to this form, in fall sowing, differs from that of Redit by a single main factor.

There is no evidence in the three crosses of linkage between the factor or factors for reaction to bunt and the factors for length of awn, color of glume, and winter or spring habit of growth.

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GROWTH OF THE COTTON ROOT-ROT FUNGUS IN SYNTHETIC MEDIA, AND THE TOXIC EFFECT OF AMMONIA ON THE FUNGUS¹

By DAVID C. NEAL, *senior pathologist*, and R. E. WESTER and KENNETH C. GUNN, *assistant scientific aids*, Division of Cotton, Rubber, and Other Tropical Plants, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The cotton root-rot disease, caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar, has been the subject of extended investigations since its discovery by Pammel (5)² in Texas in 1888. Although much knowledge has been obtained in regard to the ecology of the fungus, its life history, morphology, and some of its physiological aspects, there is as yet little known regarding the growth behavior of the pathogene under different conditions of nutrition. Several investigators have reported that the fungus grows well on a variety of media, such as cooked vegetables, various agars, plant decoctions, and sterilized roots or stems of many plants; but few attempts have been made to determine the effect of specific compounds in synthetic media on the growth of the fungus. One exception is the recent work of Ezekiel, Taubenhaus, and Fudge (1), who find that the fungus "grows readily in synthetic media" and is able to utilize both organic and inorganic nitrogen.

During the spring of 1931 the writers also studied the growth behavior of the fungus in nutrient solutions, with special attention to the nutrition of the organism with various nitrogen compounds. Comparisons were made of five different inorganic sources of nitrogen. In these studies it was found that practically no growth of the fungus occurred with ammonium nitrate or ammonium sulphate after 11, 18, and 31 days, whereas with calcium, sodium, and potassium nitrates growth was abundant. When this apparently toxic effect of ammonium salts was observed, other experiments were made to determine the effects of ammonium hydroxide and ammonia on the mycelial and sclerotial stages of the fungus. These experiments and observations form the basis of this paper.

MATERIALS AND METHODS

Duggar's solution for fungi was employed for the nitrogen comparisons, and the concentration of chemicals in the solution was as follows: M/4 dextrose, M/20 KH_2PO_4 , M/100 MgSO_4 , and a trace of FePO_4 . To this was added M/5 KNO_3 or the other nitrogen carriers having an equivalent amount of nitrogen and calculated to yield approximately 12.4 g per liter. For obtaining these dilutions the following stock solutions were prepared: M/2 dextrose, M/4 KH_2PO_4 , M/10 MgSO_4 , M/1000 FePO_4 , M/1 KNO_3 , M/2 $\text{Ca}(\text{NO}_3)_2$, M/.582 $(\text{NH}_4)_2\text{SO}_4$, and M/1.29 NH_4NO_3 . The cultures were grown in 125-cc Erlenmeyer flasks containing 50 cc of medium. They were prepared in triplicate

¹ Received for publication Nov. 16, 1932, issued August, 1933.

² Reference is made by number (italic) to Literature Cited, p

and given fractional sterilization (100° C. for one half hour for 3 days).

Owing to the fact that the fungus did not grow readily in the liquid medium when the inoculum was submerged, a hanging basket made of no. 24-gage nichrome wire was suspended in each flask to support the inoculum on the surface of the medium. By such a method, growth of the fungus may be readily obtained on any suitable nutrient solution. The flasks were inoculated after sterilization in the usual manner with a 14-day-old carrot-agar culture of the fungus, and care was taken to introduce approximately the same quantity of inoculum into each flask.

Hydrogen-ion determinations of uninoculated media in each series of cultures were made immediately after sterilization, and the dry weight of the fungous mats and the pH values of the filtrates were recorded at designated intervals, namely, 11, 18, and 31 days. At the intervals indicated the mats were collected on filter paper by means of a Büchner filter, dried to constant weight in a Freas oven, cooled for a uniform period in a calcium chloride desiccator, and weighed. The pH values were determined colorimetrically by Hellige disk comparators. The results are given in table 1.

TABLE 1.—Weight of mycelium of *Phymatotrichum omnivorum* and pH of filtrates after 11, 18, and 31 days' growth in Duggar's solution containing different nitrogen equivalents

Source of nitrogen added to Duggar's solution	Average ^a dry weight of mats, including dry weight of inoculum ^b , after—			pH value of uninoculated solution after sterilization	pH value of filtrates after—		
	11 days ^c	18 days	31 days		11 days	18 days	31 days
	Mg	Mg	Mg				
KNO ₃	282	574	451	4.3	5.3	6.0	5.8
NaNO ₃	172	651	743	4.0	5.1	5.7	5.9
Ca(NO ₃) ₂	872	974	620	3.8	4.6	4.6	5.1
NH ₄ NO ₃	84	84	153	3.9	4.4	4.4	4.3
(NH ₄) ₂ SO ₄	90	89	171	4.0	4.4	4.4	4.4

^a Averages of 3 cultures

^b Average dry weight of inoculum, 87 mg.

^c One culture only.

EFFECT OF NITROGEN FROM DIFFERENT COMPOUNDS ON GROWTH OF THE FUNGUS

It will be noted from table 1 that calcium nitrate when supplied as the nitrogen source produced the best fungous growth, which reached a maximum at the end of 18 days and declined after a longer interval, with a tendency toward degeneration of the mycelium. The next best results were obtained with sodium nitrate and with potassium nitrate. In the cultures containing the ammonium salts as nitrogen carriers no growth had occurred after 18 days, and there was only very slight evidence of mycelial growth on the inoculum in these after 31 days, when the experiment was terminated. The effects of the different nitrogen carriers on growth of the fungus in these studies are shown graphically in figure 1.

In the presence of calcium nitrate the fungus not only produced an abundant mat growth, but strand hyphae also developed above

the substratum along the walls of the flasks. In the potassium and sodium group, however, the mats were more compact, with strand hyphae almost entirely absent, and a tendency toward subsurface growth (fig. 2).

With the exception of the cultures containing ammonium salts, the filtrates became less acid as growth of the fungus progressed.

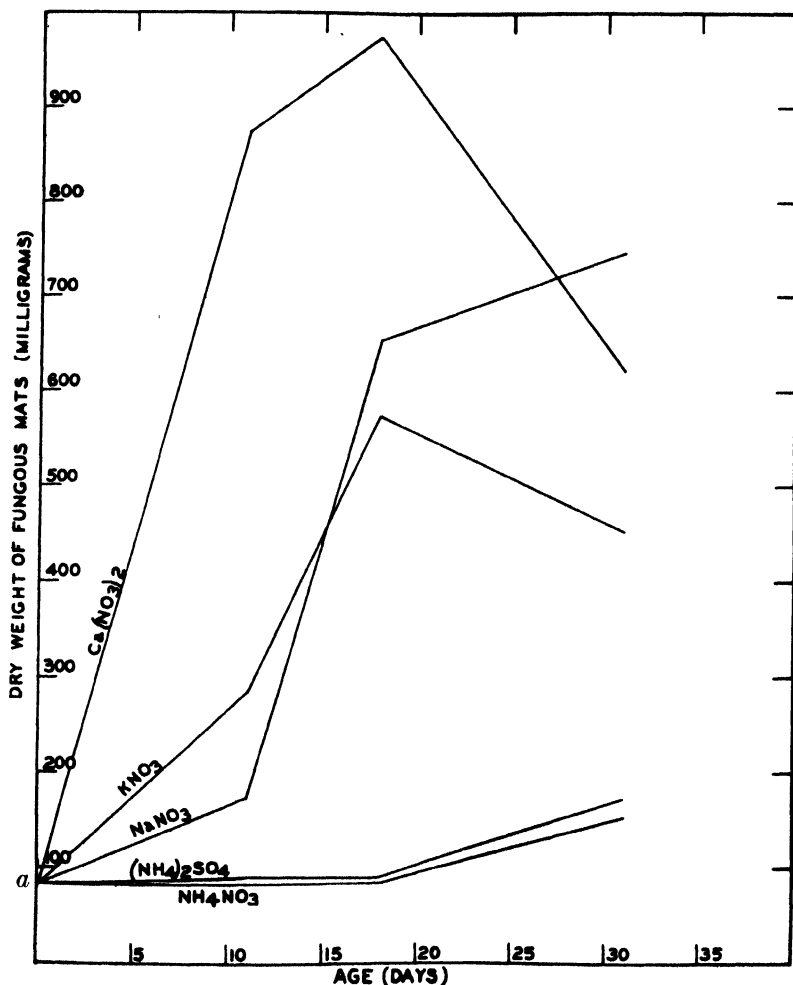


FIGURE 1.—Dry weights of *Phymatotrichum omnivorum* mats obtained in Duggar's solution with different nitrogen equivalents after 11, 18, and 31 days; a, dry weight of inoculum.

The tendency toward alkalinity in the cultures might be explained by the fact that certain fungi, unlike many bacteria, do not produce acid from carbohydrates. Waksman and Joffe (7) found that cultures of actinomycetes, when grown on NaNO_3 with the carbon source varied, showed a tendency toward alkalinity. His explanation was that the nitrate, under the influence of these organisms, is reduced to nitrite, and the liberated oxygen is united with the reducing hydrogen to form hydroxyl ions which reduce the hydron of the media.

These data indicate, therefore, that the lack of growth of the fungus in these cultures was due to the direct toxic effect of the ammonia. This has been determined by further experiments which will now be discussed.

EFFECT OF AMMONIUM HYDROXIDE AND AMMONIA ON GROWTH OF THE MYCELIUM

The procedure adopted for studying the apparently toxic effect of ammonia on the fungus was as follows: Fresh cultures were prepared in 125-cc Erlenmeyer flasks containing 50 cc of neutral carrot agar, and after growth was well established over the medium the mycelium in each of four flasks was exposed for 20 minutes to dilute ammonium hydroxide (0.1, 0.5, and 1 percent by volume, prepared from 28 percent ammonia water) by pouring 50 cc of the solution into each

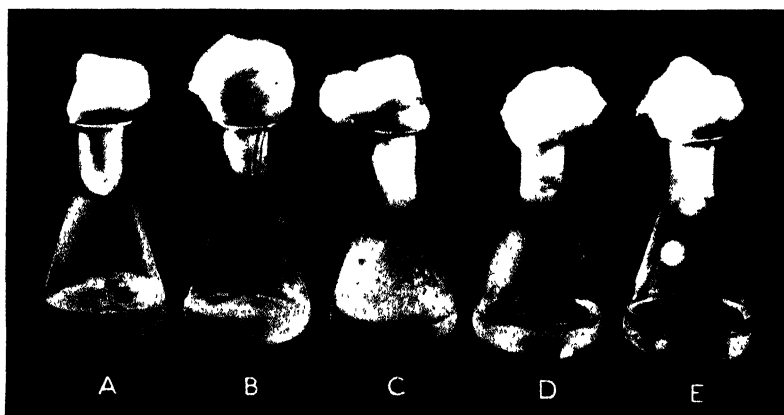


FIGURE 2. Growth of *Phymatotrichum omnivorum* in Duggai's solution with different sources of nitrogen. A, Sodium nitrate, B, potassium nitrate, C, calcium nitrate, D, ammonium nitrate, E, ammonium sulphate. Photographed after 18 days' growth.

culture. Four untreated flask cultures were reserved as checks. After the above-mentioned exposures, sectional agar blocks containing the mycelium were removed from the treated and check flasks, washed thoroughly in sterile distilled water and transferred immediately to neutral carrot-agar slants in test tubes. In every case the inoculum from the cultures treated with ammonium hydroxide failed to grow, but growth occurred in all cases from the check inoculum.

Other flask cultures were subjected to ammonia treatment for 30 seconds by generating the gas from 500 cc of 28 percent ammonia water and allowing this to enter the culture flask by means of glass tubing. This treatment also completely inhibited growth of the fungus when transfers were made. The effect of exposing the fungus to 0.1 percent ammonium hydroxide for 20 minutes and to NH_3 gas for 30 seconds is shown in figure 3.

EFFECT OF AMMONIA ON GERMINATION OF SCLEROTIA

Tests were made with root-rot sclerotia by exposing them to ammonia for short intervals (10, 15, and 20 seconds). In these tests 200 cc of 28 percent ammonia water was boiled, and the gas thus generated was allowed to enter Petri dishes containing sclerotia.

Ten sclerotia were used for each experiment, and the same number, exposed for the same intervals in sterile water, were reserved as checks. Immediately after exposure the sclerotia were transferred aseptically to neutral carrot agar and incubated at 29° C. Examinations for viability were made at intervals of 48 and 72 hours. The ammonia-treated sclerotia failed to germinate in any of the tests, but the viability of the checks was 100 percent.

The effect of ammonium hydroxide on the germination of sclerotia was also studied under laboratory conditions to determine the strength that would probably be effective for application in the field. In these experiments individual sclerotia of average size were exposed from 1 to 20 minutes in concentrations of ammonium hydroxide ranging from 0.5 to 3 percent. Twenty sclerotia were used for each exposure, and the same number, exposed for the same interval in sterile water



FIGURE 3.—Seven-day-old transfers of *Phymatotrichum omnivorum* cultures subjected to treatment with ammonium hydroxide for 20 minutes and with ammonia for 30 seconds, respectively.

only, were used as checks. The sclerotia had formed in type-soil cultures 104 days old. Immediately after exposure the sclerotia were washed in sterile distilled water, transferred to neutral carrot agar in Petri dishes, and incubated for 68 hours at 29° C. The germination results were then recorded and are given in table 2.

TABLE 2.—Effect of ammonium hydroxide on viability ^a of *Phymatotrichum omnivorum* sclerotia

Concentration of NH ₄ OH	Number of sclerotia ^b viable after exposure for			
	1 minute	5 minutes	10 minutes	20 minutes
0.5 percent	13	2	1	0
1.0 percent	8	0	0	0
2.0 percent	0	0	0	0
3.0 percent	0	0	0	0
Check ^d	20	17	19	17

^a Viability readings made after 68 hours' incubation

^b Number of sclerotia treated, 20.

^c Sclerotial mass.

^d Exposed in sterile water.

It is observed from these data that a 1 percent solution of ammonium hydroxide inhibited germination of sclerotia after an exposure of 5 minutes. The results obtained are comparable with those reported by King, Loomis, and Hope (3), who exposed sclerotia in 1 percent formalin solutions for 30 minutes and noted a reduction in germination, though some of the sclerotia survived.

EFFECT OF AMMONIA ON MYCELIUM IN INFECTED COTTON ROOTS

LABORATORY EXPERIMENTS

During the latter part of August several large root-rot-infected cotton plants were collected in the field and removed to the laboratory for chemical treatment. The tops were cut off and discarded, and the infected roots were washed thoroughly in tap water to remove the soil particles. After the surplus moisture had been removed with filter paper, the roots from each plant were placed in 500-cc Erlenmeyer flasks in duplicate and exposed to ammonia for 30 seconds and 1 and 2 minutes, respectively, the gas being generated in the manner previously described. Immediately after exposure the roots were placed in moist chambers and tested for viability of the fungus at 29° C. The ammonia treatments prevented growth after an exposure of 1 minute; good growth occurred in all the checks.

In this connection, King, Loomis, and Hope (3) studied the effect of formaldehyde gas on root-rot strands growing on moist, sterile sand in glass tubes, and found that the elongation of the strands in the tubes ceased soon after they were exposed to the gas liberated from a 1½ percent formalin solution. They report further that "root-rot sclerotia, exposed strands, and active mycelium on cotton root tissues, placed in glass tubes filled with sand, were killed when exposed for 21 hours to gas liberated from a 1½ percent formalin solution."

FIELD EXPERIMENTS

Field tests were conducted at various times during the season to determine the effectiveness of ammonium hydroxide when applied in the soil to the roots of infected cotton plants. In a preliminary test 4 percent solutions of this disinfectant did not completely inhibit growth of the fungus, consequently 6 percent solutions were used in subsequent experiments. Large mature freshly wilted plants, representing primary centers of infection, were selected and treated on August 25 with ammonium hydroxide by saturating the soil about the roots at the rate of 1 gallon per plant. Two plants were used for each test and a like number for the checks. After 48 hours' exposure the plants were removed from the soil, the roots cut off, washed, and placed in moist chambers at room temperature to test the viability of the mycelium. Observations on growth of the fungus from the tissues of the roots were made at 24-hour, 48-hour, and 5-day intervals. No growth developed from the roots of the treated plants, whereas copious development of root-rot mycelium was observed on the roots of the checks after 48 hours' incubation.

The experiment was repeated on September 22, and on this date 6 additional plants were treated with 6 percent NH_4OH as previously described and 6 were tagged as checks. The roots were placed in

moist chambers 40 hours after treatment, and growth observations were recorded after 48 hours and 5 days. With one exception, the mycelium was killed in the tissues of the roots of plants treated with

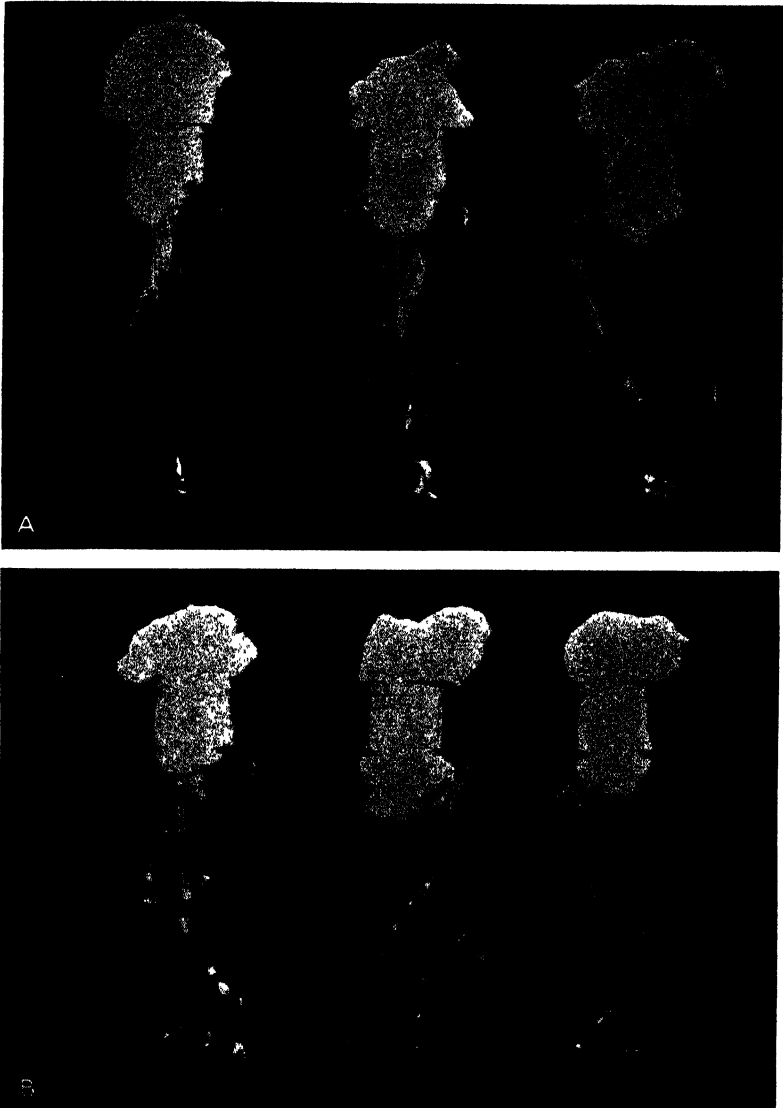


FIGURE 4.—Effect of field applications of ammonium hydroxide on roots of cotton plants infected with *Phymatotrichum omnivorum* after being in moist chamber at 29° C. for 5 days: A, Roots from infected plants treated for 48 hours with 6 percent ammonium hydroxide. Note absence of *Phymatotrichum* mycelium. B, Check, roots from infected plants.

6 percent ammonium hydroxide (fig. 4), and in the single case where growth actually developed it was noticeable only to a slight extent after the maximum period of incubation.

EFFECT OF AMMONIUM HYDROXIDE ON COTTON PLANTS

In the preliminary tests with ammonia, used in disinfecting the roots of infected cotton plants in the field, it was observed that in the majority of cases 4 percent solutions of the hydroxide did not kill the plants. On the other hand, 2 percent solutions of formalin frequently caused wilting in 3 or 4 days, and in many instances considerable defoliation resulted.

In order to gain further information as to what concentrations of the ammonia water would be likely to prove sufficient to kill cotton plants under field conditions, and at the same time serve as a guide for experimental application for various ornamental plants, additional experiments were made. Plants were treated during August, September, and October 1931, with 4, 6, and 8 percent solutions, respectively, of ammonium hydroxide by saturating the soil, as previously indicated, about the roots at the rate of 1 gallon per plant. Afterwards observations were made each day for several weeks. The plants treated with 6 percent solutions survived the treatment (fig. 5), notwithstanding the fact that considerable injury resulted in the cortical and cambium tissues of the roots (fig. 6). The plants that received 8 percent solutions, however, were noticeably affected; 50 percent being killed within 7 to 9 days after treatment (fig. 5).

DISCUSSION

In interpreting the toxicity of ammoniacal nitrogen in the case of higher plants, other workers (4, 6) have advanced the hypothesis of physiological acidity or increasing hydrogen-ion concentration as a partial explanation. However, the marked suppression of growth of the fungus in the presence of the ammonium compounds in these experiments does not appear to be attributable to the acid reaction of the solutions, inasmuch as the other nitrogen carriers in the uninoculated flasks also revealed considerable acidity, as is indicated by the pH values after sterilization.

The effect of ammonia in preventing growth of the fungus, as indicated in the experiments reported in this paper, may explain some of the discrepancies encountered in earlier field applications of manure for the control of root rot. In some cases root-rot losses have been definitely prevented by continued applications of barnyard manure (2), whereas in other cases no restriction of root rot has resulted. The effect of manure upon the root rot may depend upon the early or ammoniacal decomposition of the manure; this would be in accord with field experiments in which applications of ammonia killed the root-rot mycelium but did not kill the cotton plants.

The possibility of utilizing ammonia or ammonium-yielding compounds for control of the disease in cotton fields as well as for protecting ornamental plants or shade trees is suggested, and field experiments to determine to what extent the ammonium treatment can be applied to general farm practice are now in progress.

SUMMARY

The cotton root-rot fungus, *Phymatotrichum omnivorum*, was grown in Duggar's solution for fungi, and growth comparisons were made of five inorganic sources of nitrogen.



FIGURE 5.—Lone Star cotton plants after treatment with ammonium hydroxide, 1 gallon per plant: A, 6 percent, photographed after 60 days; B, 8 percent, photographed after 9 days; C, check.

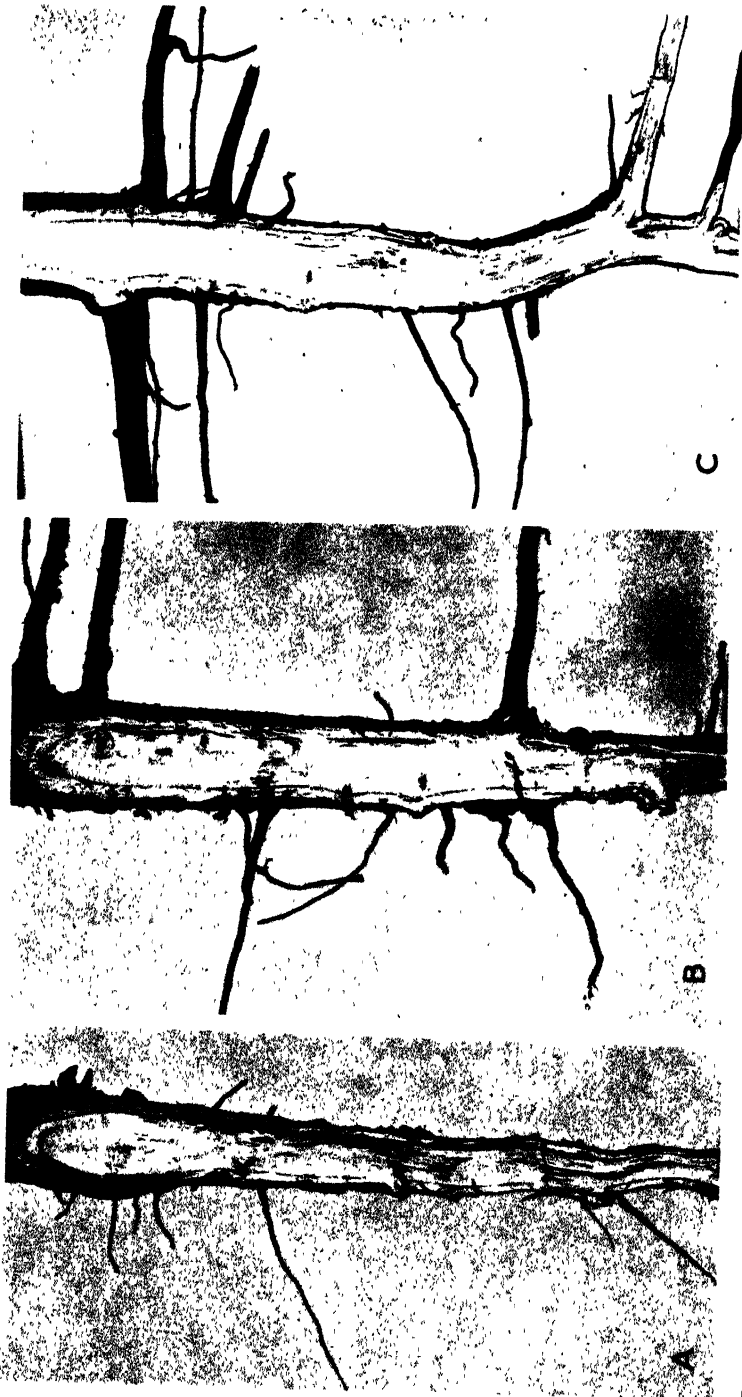


FIGURE 6 —Roots of Lone Star cotton plants after treatment with ammonium hydroxide, 1 gallon per plant
graphed after 9 days. C, check

A, 6 percent, photographed after 60 days; B, 8 percent, photo-

With the nitrogen equivalents used at a concentration to yield approximately 12.4 g of nitrogen per liter, very little growth of the mycelium appeared after intervals of 11, 18, and 31 days with ammonium nitrate or ammonium sulphate, whereas with calcium nitrate, sodium nitrate, and potassium nitrate, abundant growth was produced.

Growth characters of the fungus obtained with the various nitrogen sources are described, and the toxic action of ammonium compounds is discussed.

With the exception of the cultures supplied with ammoniacal nitrogen, pH exponents of the filtrates increased as growth of the fungus progressed. The probable cause of the shifting of hydrogen-ion concentration of the media is given.

The apparent toxic effect of ammonia on the fungus was confirmed by subsequent tests, the mycelium being killed with ammonium hydroxide at a concentration as low as 500 parts per million after an exposure of 20 minutes. Gas liberated from 28 percent ammonia water also killed the mycelium in 30 seconds.

Ammonia inhibited germination of root-rot sclerotia after exposures as short as 10, 15, and 20 seconds. Sclerotia were killed in 5 minutes by 1 percent solutions of ammonium hydroxide.

Growth of the mycelium from root tissues of infected cotton plants was prevented by exposure to ammonia for 1 minute in laboratory tests. In field tests 6 percent solutions of ammonium hydroxide applied to the soil around the roots of infected cotton plants killed the mycelium in most cases, whereas abundant growth developed from the checks.

In experiments conducted in the field to determine the effect of ammonium hydroxide on cotton plants, it was found that mature plants were not killed with 4 or 6 percent solutions, although the cortical and cambium tissues of the roots were injured. The lethal effect of 8 percent solutions, however, was pronounced.

The inhibiting effect of ammonia on growth of the fungus and the probable relation this may have to actual control of the disease through continued applications of barnyard manure are discussed.

The possible utilization of ammonia or ammonium compounds for the control of the disease in cotton fields and for protecting ornamental plants is suggested by the experiments reported.

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DISTRIBUTION AND RATE OF FALL OF CONIFER SEEDS ¹

By HOWARD W. SIGGINS ²

Late associate silviculturist, California Forest and Range Experiment Station,
Forest Service, United States Department of Agriculture

INTRODUCTION

The first concern of American silviculture in its present stage of development is to secure satisfactory stands of young trees to take the place of mature trees which have been cut or are about to be cut. Where reliance is placed upon natural regeneration, the seeding habits of the species involved are matters of fundamental importance. For species which depend upon the wind for transportation of their seed from the parent tree to the place where the young tree is to grow, an understanding of the factors governing wind distribution of seeds would appear to be essential. The majority of the most important timber species of the world bear seeds adapted to wind dissemination, but a search of forestry literature antedating 1925 fails to bring to light any material of definite value on this subject. Most of the observations which have been made (1, 3, 4)³ seem to have been based on studies of existing reproduction, accompanied by estimates (or guesses) as to the probable source of seed supply. In the few cases where the seed source was definitely known the meteorological side of the problem has remained untouched. No attempt appears to have been made to measure the rates of fall of each species of seed and thus determine how far it will be carried by winds of given velocities. Since 1925, however, several popular and semipopular articles in trade journals and other publications (2, 6, 7, 13, 16) have touched on other phases of the subject, particularly several written by Isaac (10, 11, 12), who began the study of this problem about the same time as the writer.

SEED CHARACTERISTICS WHICH AFFECT THE RATE OF FALL

Seeds which are adapted to dissemination by wind vary greatly in size, weight, and shape, in area and conformation of wing, and in combinations of these seed and wing qualities. In general one may say that species producing heavy seeds tend to have a faster rate of fall than species producing light seeds. However, weight alone is not the determining factor (14, 15). This is shown by the fact that seeds of *Chamaecyparis thyoides* (L.) B.S.P. averaging 0.0016 g in weight fall more rapidly than do seeds of *Pinus lambertiana* Dougl. averaging 0.293 g. Size of wing alone is no more valuable as a measure of rate of fall. Seed of *Abies amabilis* (Loud.) Forb. falls nearly twice as fast as seed of *Tsuga heterophylla* (Raf.) Sarg., though the former has by far the greater wing area.

¹ Received for publication Oct. 17, 1932; issued August 1933.

² In the original manuscript, prepared by Mr. Siggins just before his death in September 1929, he expressed grateful acknowledgments to the many persons who assisted him in this study by helpful suggestions or by collection of materials, and especially to Woodbridge Metcalf, associate professor of the University of California and extension forester of the State of California, with whose advice and assistance the study was begun in 1925.

³ Reference is made by number (italic), to Literature Cited, p. 127.

A falling seed usually whirls around its center of gravity. This whirling is caused by the curvature of the wing. Since the amount of curvature varies greatly within any one species, the rate of whirling also varies (fig. 1). A few seeds of each species seem to have straight

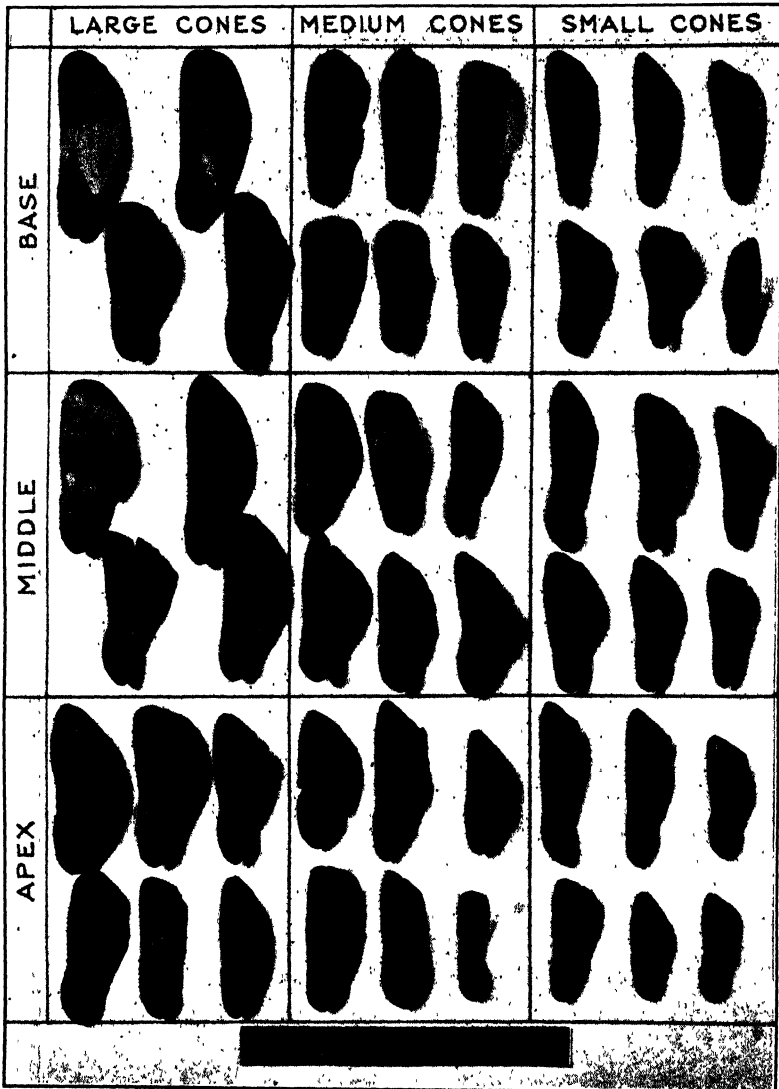


FIGURE 1.—Variability of sugar pine seeds from different cones and from different parts of each cone. Seeds are arranged vertically by size of cone: Left, large cones; middle, medium cones; right, small cones. Horizontally by location in cone: Top, base of cone; middle, midcone; bottom, apex of cone.

wings which do not produce a whirling motion. Such seeds fall very rapidly.

No clear-cut classification of seeds according to characteristics of wind resistance seems to be possible. From actual observation, however, one may say that—

(1) Seeds with marginal wings, such as redwood or birch, fall very rapidly for their weight. In these seeds the center of gravity corresponds closely to the center of surface area.

(2) Seeds with short broad terminal wings, such as the firs, have a less rapid rate of fall for their weight. In these seeds the center of surface area is somewhat removed from the center of gravity, though not far from the wingward end of the long seed.

(3) Seeds with terminal wings much longer than their width, such as the pines and spruces, fall least rapidly for their weight. In these seeds the center of surface area is considerably removed from the center of gravity.

From these observations one might advance the hypothesis that the efficiency of a seed wing, in supporting a given weight, is proportional to the distance between the center of gravity and the center of surface area (fig. 2). Since the seed whirls about its center of gravity, this distance becomes the radius of the circle described by the center of surface area. Inasmuch as the circumference is proportional to the radius, it might be said that the efficiency of the wing in supporting a given weight is proportional to the distance traveled by the center of wing area. Such a statement involves another factor, however, namely, the number of times the wing whirls about the seed in falling a given distance. A long and detailed study would be necessary to prove this hypothesis. No attempt was made to

do so, because the only results of practical value have been more simply attained by measuring the average rate of fall for each species.

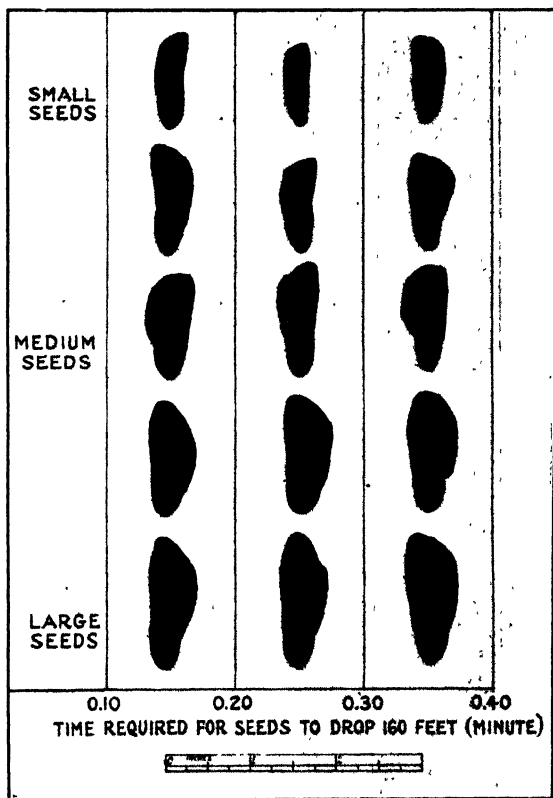


FIGURE 2.—Relation of seed and wing size in sugar-pine seeds having equal rate of fall

MEASUREMENT OF RATE OF FALL

The first attempts to determine rates of fall in 1925 were made by liberating seeds from an elevation of 100 feet on a tall flagpole on the military parade ground of the University of California. The results obtained were highly variable. The next step was to release toy balloons with the seeds in order to be able to follow the flight more

easily. A given balloon should naturally have a constant rate of fall in quiet air. The balloons released with the seeds, however, showed the same variations in rate of fall as did the seeds. Furthermore, it was observed that the balloons sometimes followed an irregular line in descending. The solution was now apparent. Drilling students had trampled out the grass surrounding the flagpole and in several other spots. Convectional air currents, rising over the sun-warmed bare soil and descending over the large patches of cool sod, were affecting the rates of fall. Variations in direction of the wind during the 98 tests made at this place resulted in wide variations in the air conditions to which the falling seeds were subjected. The extent of these variations is shown in table 1.

TABLE 1.—Range of rates of fall (feet per second) in rapid and slower falling lots of seeds of different species, showing the effects of convectional currents and other turbulence

Species	Most rapidly falling lot		Least rapidly falling lot	
	First seed	Last seed	First seed	Last seed
Sugar pine	18.5	4.4	6.7	2.0
Redwood *	11.1	6.7	9.3	4.8
Ponderosa pine	8.3	3.5	3.1	1.2
White flr.	11.1	5.6	4.8	1.1
Red flr.	9.3	3.5	8.3	2.6
Grand flr.	16.7	9.3	3.3	2.4
Sitka spruce	3.7	1.8	(^b)	(^b)
Douglas fir	5.6	2.7	2.8	.7
Incense cedar	8.3	2.8	(^b)	(^b)

* Redwood seeds are so small that they were impossible to follow in the air and very difficult to find in the grass. The maximum rates are probably too low.

^b 6 lots of Sitka spruce and 1 lot of incense cedar were caught by ascending convectional currents and carried upward out of sight

The next step was to find a place in which to conduct trials in undisturbed air. An ideal laboratory for this purpose was at hand in the campanile, or bell tower, on the University of California campus. This structure is a masonry shaft 302 feet high and 33 feet square at the base. It is divided into compartments by floors, the first at 30 feet from the ground and the successive floors every 25 feet above. In the center of the tower is a concrete elevator shaft, about 7 feet square inside. This runs from a pit 10 feet below the ground floor to the landing at the 180-foot level. The openings to the elevator at each floor are closed with solid oak doors. With all doors closed the shaft becomes a well-insulated chamber in which temperature conditions change very slowly and no air movement is detectable with a Biram anemometer.

The seeds were dropped from a trap held in the center of the shaft at the next-to-top landing and were caught on muslin screens laid on a concrete pier 5 feet high in the center of the pit. This gave a clear fall of 160 feet. The screens had to be limited in size and number because of the crowded conditions in the pit and the necessity for quick and certain manipulation. Ten screens 3 feet square were used. The first screen was in position when the releasing trap was opened. A second screen was laid on top of the first when the first whirling seeds appeared. Thereafter additional screens were superimposed every 0.1 minute. One man measured the time with a stop watch while a second man manipulated the screens. Occasionally mechanical difficulties caused slight errors in the time at which screens were

laid down, but the error was rarely as much as 1 second. Except when the mode of the distribution occurred near the time of changing screens, this error did not greatly affect the calculated mean.

After the seeds of a lot had all fallen the sample collected on each screen was transferred to a paper bag for later analysis. Table 2 is a typical analysis of one lot of seeds.

TABLE 2.—*Distribution by rate of fall of one lot of loblolly pine seeds dropped 160 feet in quiet air*

Time of falling (minutes)	Total seeds		(good seeds ^a)		Weight of good seeds	
	Number	Percent	Number	Percent	Total	Average
0 10-0 20.....	13	1.7	10	1.8	0.32	0.032
0 20-0 30.....	5	.7	5	.9	.17	.034
0 30-0 40.....	3	.4	2	.3	.06	.03
0 40-0 50.....	4	.5	1	.2	.03	.03
0 50-0 60.....	96	12.6	76	13.3	2.40	.032
0 60-0 70.....	376	49.3	324	56.7	10.70	.033
0 70-0 80.....	200	26.2	122	21.4	4.06	.033
0 80-0 90.....	43	5.6	23	4.0	.75	.033
0 90-1 00.....	15	2.0	8	1.4	.26	.032
1 00-1 10.....	8	1.0	0	0	0	0
Total.....	763	100.0	571	100.0	18.75	.033

^a 75 percent of the seeds are good The average good seed falls 160 feet in 0.6567 minute, 244 feet in 1 minute, or 4.1 feet in 1 second

Table 2 shows that some seeds descend much more rapidly and some much more slowly than the mean rate of fall. The great bulk of the seeds, however, fall in a rather concentrated group. This concentration about the mean rate of fall is typical of all lots of seeds. Different samples of seeds of the same lot show differences in the calculated mean rates of fall, but these differences are well within the limits of experimental error.

TABLE 3.—*Percentage distribution by rate of fall of Pinus taeda seeds from different trees and localities when dropped 160 feet in quiet air*

Time of falling (minutes)	Seeds from Virginia							Seeds from Texas
	Tree no 2	Tree no 3	Tree no 1	Tree no 10	Tree no 11	Tree no. 5	Tree no 12	
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
0-0 30.....	6.0	2.4	3.3	2.6	9.9	4.5	6.1	5.6
0 30-0 40.....	1.4	.8	1.0	.4	1.9	1.1	3.5	1.2
0 40-0 50.....	2.0	1.2	2.4	.2	1.5	1.4	6.3	48.9
0 50-0 60.....	3.8	7.0	2.9	13.3	11.0	45.9	58.8	28.1
0 60-0 70.....	14.4	40.6	59.3	56.7	36.0	31.8	22.0	10.6
0 70-0 80.....	48.1	43.9	21.8	21.4	33.2	10.5	2.5	3.3
0 80-0 90.....	20.6	4.1	6.3	4.0	5.8	4.1	.8	1.3
0 90-1 00.....	3.1	0	2.2	1.4	.7	.4	0	.8
1 00-1 10.....	.6	0	.8	0	0	.2	0	.2
1 10-1 20.....	0	0	0	0	0	.1	0	0
Average weight of good seeds.....	Gram 0.031	Gram 0.023	Gram 0.035	Gram 0.033	Gram 0.021	Gram 0.021	Gram 0.030	Gram 0.026
	Feet per second	Feet per second	Feet per second	Feet per second	Feet per second	Feet per second	Feet per second	Feet per second
Average rate of fall.....	3.8	3.9	4.0	4.1	4.2	4.5	4.9	5.3

One group of tests calling for special mention is the series in which *Pinus taeda* seed was used. The Appalachian Forest Experiment Station sent seven lots of loblolly pine cones collected in Virginia, each lot containing cones from only one tree. The calculated rates of fall,

obtained from one test with each lot of seed, were 3.7, 3.9, 4, 4.1, 4.3, 4.4, and 4.9 feet per second. The mean rate was 4.2 and the median rate 4.1 feet per second. These figures show that the results of the rather limited number of tests here reported cannot be considered as exact and invariable quantities. Furthermore, a lot of loblolly cones sent by the Southern Forest Experiment Station from Texas showed a decidedly different rate of fall, 5.3 feet per second, suggesting that seeds of the same species may develop regional characteristics which require study. Table 3 shows the results of these tests in more detail.

There seems to be some evidence of slight negative correlation between weight of seed and rate of fall in the eight lots of the species shown in table 3. The fastest falling seeds are lighter than the average weight. The heaviest seeds fall more slowly than the average. The negative correlation shown by different lots of this species apparently is contradictory to the general tendency in the genus *Pinus* for the rate of fall to be positively correlated with the average weight of the seed, as indicated by the data in table 4.

TABLE 4.—Average rate of fall and average weight of seed in 12 species of pine

Species	Source	Average rate of fall	Average weight
		Feet per second	Gram
<i>Pinus lambertiana</i> Dougl.	California	8.7	0.293
<i>Pinus jeffreyi</i> "Oreg. Com "	do.	7.25	.135
<i>Pinus pulustris</i> Mill.	Louisiana	5.8	.120
<i>Pinus ponderosa</i> Laws	California	5.0	.058
<i>Pinus caribaea</i> Mor.	Florida and Louisiana	4.6	.042
<i>Pinus taeda</i> L.	Virginia	4.2	.027
<i>Pinus echinata</i> Mill.	Louisiana and Texas	3.85	.012
<i>Pinus balfouriana</i> Murr.	California	3.7	.024
<i>Pinus strobus</i> L.	North (?) Carolina	3.3	.024
<i>Pinus virginiana</i> Mill.	do.	2.85	.010
<i>Pinus pungens</i> Lamb.	do.	2.8	.013
<i>Pinus contorta</i> Loud.	Wyoming	2.7	.0055

Seeds of other genera do not show the same correlation between weight and rate of fall as do seeds of the pines. Table 5 gives figures on some of the other species which have been tested.

TABLE 5.—Average rate of fall and weight of seed of species other than pine

Species	Source	Average rate of fall	Average weight of good seeds
		Feet per second	Gram
<i>Abies grandis</i> Lindl.	California	5.7	0.024
<i>Abies concolor</i> Lindl. and Gord.	do.	5.7	.070
<i>Abies magnifica</i> Murr.	do.	5.3	.110
<i>Abies fraseri</i> (Pursh) Poir.	North Carolina	5.1	.013
<i>Abies amabilis</i> (Loud.) Forb.	Oregon	4.95	.075
<i>Betula lutea</i> Michx.	North Carolina	5.1	.0013
<i>Chamaecyparis thyoides</i> (L.) B.S.P.	Virginia	9.6	.0016
<i>Libocedrus decurrens</i> Torr.	California	5.9	.065
<i>Liriodendron tulipifera</i> L.	North Carolina	6.2	.056
<i>Picea rubra</i> Link.	do.	3.9	.0053
<i>Picea sitchensis</i> (Bong.) Carr.	California	3.1	.0034
<i>Pseudotsuga taxifolia</i> (Lam.) Britt.	do.	4.4	.017
	Colorado	4.4	.009
<i>Sequoia sempervirens</i> (Lamb.) Endl.	California	8.6	.0074
<i>Thuja plicata</i> D. Don	Oregon	5.1	.0015
<i>Tsuga heterophylla</i> (Raf.) Sarg.	do.	2.6	.0021

DISTRIBUTION OF SEEDS FROM THE CONE

Any group of seeds liberated from one tree at the same instant will tend to be distributed in a straight line to leeward of the tree. If all seeds produced by a tree were to be released at one time the result would be an undesirable concentration of seeds in a limited area and a total lack of seeds in other areas. The mechanisms of dissemination are such, however, as to provide a continuing supply of seeds over a considerable period (fig. 3). A pine cone, for example, opens one scale at a time. As the scale opens the two seeds which were borne under it are permitted to drop out. Ordinarily several days elapse between the escape of the first and last seeds from any one cone. Furthermore, the cones on different parts of the same tree do not all open at the same time. One tree may be casting seed for several weeks. Variations in direction and velocity of the wind during this period result in widespread scattering of the seeds. Where the winds are rather consistently from one direction the area seeded will be in the form of a V, with the apex at the base of the tree. The areas of the concentric radial zones increase as the squares of the radii. The greatest number of seeds per square foot of ground will therefore be found quite close to the base of the tree. The concentration of seeds will then decrease rather gradually with increasing distance from the tree until the point is reached where the average seed is dropped by the wind of average velocity. Beyond this point the concentration of seeds will diminish very rapidly.

In a virgin forest the supply of seed is so great and so uniformly distributed that there is no significance to this question of how far seeds are carried. However, it is important when considering the seeding of a cut-over area from a body of uncut timber to windward. The distance of seeding from the uncut timber is controlled by the velocity of the wind blowing at right angles to the edge of the cutting.

A forester who wishes to plan carefully the distribution of seed trees on a cut-over area must consider not only the velocity but also the variability of direction of winds which are to distribute the seeds. The ideal condition would exist when winds might be expected to blow from all directions. Each tree would then stand in the center of a circle and would seed the maximum area without overlapping. Where the prevailing winds come principally from one direction, however, the seed distribution will be in long narrow V's and considerable overlapping will be necessary to provide for completely covering the ground.

While the question of quantity of seed required might not seem to be pertinent, it may be well to mention the subject as a matter of caution. The destruction of seeds by birds and rodents is so great that a distribution of seed trees sufficient to cover the area with a satisfactory concentration of seeds might prove entirely inadequate for securing a good stand of reproduction. Taylor⁴ reports that caged ground squirrels ate on an average 340 seeds of ponderosa pine in one night. Chipmunks ate 237 pine seeds in the same time. Four seed trees per acre, averaging 92,000 seeds per year, he says—

would be insufficient to do more than win the grateful appreciation of the resident squirrels and chipmunks were it not for the young pines that actually do come in on these cut-over areas in defiance of biological statistics.

⁴ No comprehensive report of the findings of Walter P. Taylor in the Southwest has been published.

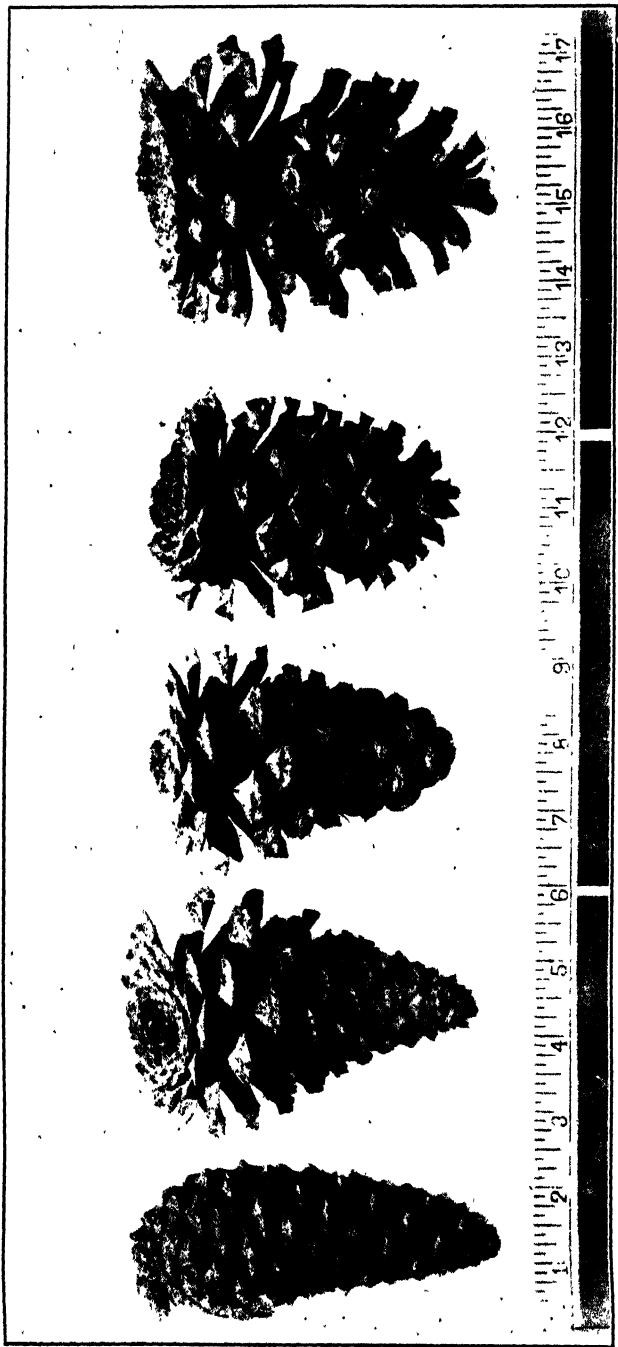


FIGURE 3.—Progressive steps in the opening of a ponderosa pine cone

These trees, of course, arise from seeds which have fallen where they escaped the search of the rodents and birds or from seeds which have been buried and forgotten by the little beasts.

Because of this destruction of the seed crop, many trained investigators have been willing to accept such statements as "seed trees should be so spaced that no tree would be depended on to seed to a distance greater than the height of the tree." It is now evident that at least a part of the reproduction which is found at long distances from any seed trees may safely be attributed to wind-blown seeds. In the case of large, severely burned areas, the bulk of the rodents are probably killed by the fires or die of starvation or disease shortly afterward. This renders the areas comparatively safe for the seeds which fall upon them, and the rate of establishment is high when soil and moisture conditions are favorable.

SUMMARY AND CONCLUSIONS

Each species of tree produces seeds having a characteristic average rate of fall, although this rate may vary considerably with the region in which the tree is grown.

Because of the range in rates of fall of seeds from one source and variations in the height at which the seeds are produced and in the direction and velocity of winds which distribute these seeds, it is not possible to predict exactly the distribution of seeds which will be obtained from a given seed tree in any one season. The total distribution over a period of years can be predicted with reasonable accuracy, however, if the meteorological conditions of the locality are carefully studied.

Forest-tree seeds adapted to dissemination by wind are carried to much greater distances than was previously supposed, but under normal conditions the dissemination of tree seeds to great distances is of small importance to the silviculturist.

Under certain unusual conditions the eventual reforestation of extensive burns may be assured by trees grown from seeds carried for many miles. Such seeds may number only one in a million, but the great number of seeds produced renders the aggregate of extreme importance in such cases.

The effective distribution of seeds is much smaller than the actual distribution because of the destruction of seeds by rodents. Rodent-control measures should greatly reduce the economic loss caused by the present necessity of tying up large amounts of lumber in seed trees. Until this is accomplished, the quantity of seed produced per acre, rather than the distance to which it is distributed, must be the criterion for determining the number of seed trees to be left for reforestation of cut-over lands.

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YIELD AND PROTEIN CONTENT OF HARD RED SPRING WHEAT UNDER CONDITIONS OF HIGH TEMPERATURE AND LOW MOISTURE¹

By L. R. WALDRON²

Agronomist (plant breeding), North Dakota Agricultural Experiment Station

INTRODUCTION

This paper reports a statistical study of yield and other characters of hard red spring wheat grown under conditions of high temperature and low moisture, of a light incidence of disease, and on a fertile soil known as Fargo clay. Nearly all the wheats were chosen because of high yield, when grown under certain environmental conditions, and most of them were selected for resistance to stem rust. Relationships were studied by the use of means, standard deviations, and standard errors, and the analysis was continued by a consideration of total and partial correlation coefficients, by coefficients of multiple determination, and by total and partial regression coefficients. An analysis is attempted of a relative study of yield and protein content.

EXPERIMENTAL PROCEDURE

This experiment, conducted at Fargo, N.Dak. in 1932, was 1 of a uniform series of 18 such experiments in this hard red spring wheat area and consisted of 25 varieties planted in a randomized manner in triplicated and guarded rod rows. At harvest time 3-foot samples were pulled from the guard rows for detailed study. These samples were selected as to uniformity of stand, care being taken that no empty spaces in the rows were included. In the case of three of the varieties the guard rows were planted with sib material and in those cases the 3-foot samples were taken from the center or yield rows. The detailed study upon the seventy-five 3-foot samples included the following items: Total number of plants and of heads and the calculated stooling; the number of fertile, sterile, and total spikelets per spike; the total number of kernels per spike and the number of third or mid kernels in the spikelets for each spike; the weight of the grain for each 3 feet, the weight of grain per spike, and the weight per 1,000 kernels; the plumpness and percentage of yellow berry of the kernels; and finally the percentage of protein. In addition to the laboratory data secured on the 3-foot samples, the usual field notes were taken on the standing crop at and before the time of harvest.

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² Acknowledgment is made by the author to A. J. Pinckney, assistant agricultural chemist, North Dakota Agricultural Experiment Station, for the analyses made upon wheat samples for protein content, also to the 18 regional nurseries, of which this was one, in which a series of experiments was carried on cooperatively with the U. S. Department of Agriculture.

WEATHER DATA

Enough weather data are presented to aid somewhat in the interpretation of the experimental results. The normal mean and 1932 mean temperature and precipitation data for the 4 months, April to July, are shown in table 1.

TABLE 1.—Normal mean and 1932 mean temperature and rainfall data for each of 4 months at Moorhead, Minn.^a

Year	April		May		June		July	
	Mean temperature	Rainfall	Mean temperature	Rainfall	Mean temperature	Rainfall	Mean temperature	Rainfall
	° F.	Inches	° F.	Inches	° F.	Inches	° F.	Inches
Normal.....	41	2 18	55	2 89	64	4 05	68	3.43
1932.....	41	1 06	57	2 05	69	2 16	72	1 36

^a Data are from the official records of the United States Weather Bureau

The drought of 1932 was really a continuation of a drought persisting for at least 4 years. The total deficiency in rainfall for the 4 months indicated for the 4 years 1929 to 1932 was upwards of 19 inches. As the subsoil was very dry in 1932, the amount of moisture depended almost entirely on the showers during the growing season. It is worth noting that during 18 days in July the excess in temperature totaled 155° F.

EXPERIMENTAL DATA

The wheats and their yields as determined from the rod rows and from the 3-foot rows are shown in table 2.

TABLE 2.—Yields of wheat as determined from 3-foot rows and from rod rows

No.	Variety	Yield (bushels per acre)	
		3-foot row	Rod row
12	Kota × Webster, H-209.....	46.1	46.4
8	Marquis × Kota, 1656 106.....	43.4	46.2
21	Marquis × Hard Federation, 649.....	41.8	44.4
10	Ceres (Marquis × Kota).....	41.8	44.3
7	Marquis-Kota × Hope, Ns 2421.....	42.6	42.9
9	Marquis × Kota, 1658 178.....	47.9	42.0
19	Minnesota 2340 (Marquis-lumillo × Kanred-Marquis).....	36.0	40.1
16	Minnesota 2315 (Marquis-lumillo × Kanred-Marquis).....	35.2	39.5
6	Hope × Florence, Ns. 2503.....	44.0	39.3
4	H-44 × Marquis, R L 592.....	36.3	38.6
2	Hope × Ceres, N.N. 1098.....	41.9	37.5
1	Marquis.....	31.8	36.7
20	Marquis × Emmer, H-44.....	36.9	35.9
5	Pentad × Marquis, R.L. 729.....	36.2	35.7
11	Kota × Webster, H-151.....	39.0	35.5
14	Marquis-Emmer × Marquis, Sel. 1280.....	34.9	35.4
22	Marquis × Hard Federation, 666.....	37.3	35.0
15	Marquis-Emmer × Kota-Webster, N N. 1087.....	41.3	34.1
25	Hope × Reliance, N.N. 1131.....	31.9	33.8
18	Minnesota 2302 (Marquis-lumillo × Kanred-Marquis).....	36.7	33.3
17	Minnesota 2316 (Marquis-lumillo × Kanred-Marquis).....	34.3	32.7
3	Hope × Ceres, N.N. 1127.....	26.6	31.4
23	Hope × Reliance, N.N. 1121.....	31.2	30.8
24	Hope × Reliance, N.N. 1128.....	28.8	30.4
13	Marquis-Emmer × Marquis, Sel. 1087.....	32.7	23.8
Average.....		37.4	37.0

^a Below this point odds are 23 to 1 or more against the occurrence by chance of deviations as great as, or greater than, the ones indicated from the highest yield.

All wheats in table 2 are unnamed hybrid selections except two, Marquis and Ceres. The variety Hope, entering into several of the crosses, was produced by McFadden (5)³ by crossing Marquis and emmer. This variety and also H-44 are highly resistant to both kinds of rust and smut.

The resistance of Hope to stem rust is remarkable in that the plant in the mature stage in the field is not appreciably attacked; it must thus be resistant to all physiological forms present. This variety also shows resistance to leaf rust. Smith (8) has shown that Hope when seeded in the spring is highly resistant to five physiologic forms of *Tilletia tritici* and *T. levis* but is moderately susceptible to the same forms when planted in the fall. Of the 25 wheats in the nursery 14 had a 14-chromosome wheat parent either as P₁ or P₂. With the exception of Marquis and the two wheats derived from the cross Marquis × Hard Federation, selection has been made for resistance to stem rust. The wheats in this experiment were not affected appreciably by disease except that the yield and bushel weight of the Marquis-Hard Federation cross no. 666 were reduced by stem rust. Injury of this sort to the companion selection no. 649 was decidedly less and probably not at all material. The essential absence of disease in an injurious form simplified the study.

Nearly all the wheats in the experiment were included because of promising performance in yield. The great range of yields in the rod rows, from 46.4 to 23.8 bushels, indicates that in the essential absence of losses due to disease some other factor or factors must have been operating to reduce yields of certain varieties. Because of the drought and high temperatures prevailing, it is likely that it is these factors to which one must look for an explanation of certain low yields. This paper presents a study of a series of wheats subjected to high heat and low moisture, factors which exerted differential effects in regard to yield. Yield represents a complex of contributing components.

Aside from those shown in table 2, yields were taken of one of the guard rows. In three instances the guard rows were of sib selections thus bringing an error into the guard-row yields. The guard rows averaged 37.5 bushels per acre, a difference of but one half bushel from the center, or yield rows. The correlation in yield between the center and the one series of guard rows was 0.652. There was no difference in variability of yield between the two series of rows, guard and center. This indicates that adjacent border rows of different varieties had not acted as an upsetting factor in border-row yield. The correlation in yields between the 3-foot rows, taken from the other guard rows, was 0.659 which is essentially the same as the coefficient just cited. The variability in yield of the 3-foot samples was not significantly greater than the similar coefficients from the rod rows. The yields from the 3-foot rows evidently corresponded fairly well with the yields from the longer rows. Most of the data were secured from the shorter rows, and thus yields and other characters are generally in close correspondence.

³ Reference is made by number (*italic*) to Literature Cited, p. 147.

The variety Hope and its sibs have shown rather marked susceptibility to hot and dry weather, and this character has been exhibited in a considerable proportion of the offspring. From table 2 it is evident that hybrids from the Marquis-Emmer parentage tend to yield less than the average of the group. The wheats of this particular hybrid origin yield very significantly less than the wheats unrelated to Hope, when averages are compared, in the group under consideration. Ceres and its allies have shown a capacity to produce relatively well under conditions of drought and heat (Waldron (10)). Wheats of other relationships are shown among the 25, and this study may be of aid in showing which physical characters are associated with high and low yields under medium drought conditions.

Most of the studies presented in this paper are based upon correlation and regression data. Table 3 shows the averages of groups of 5 of the 25 wheats, arranged in order with respect to yield, and to 9 other characters of greater or less importance. Similar data are presented for the highest-yielding and the lowest-yielding wheats and for the two named varieties, Ceres and Marquis.

TABLE 3.—Data on 25 wheats segregated into 5 groups and on 4 individual wheats showing means as indicated

Group no	Yield per acre	Heads per row	Kernels per head	Mid kernels per head	Weight per 1,000 kernels	Weight of grain per head	Plumpness (scale 8-1)	Height	Date first heads	Protein in wheat
	<i>Bushels</i>	<i>Number</i>	<i>Number</i>	<i>Percent</i>	<i>Grams</i>	<i>Centigrams</i>		<i>Inches</i>		<i>Percent</i>
1	44.8	130	27	12.5	33.7	65	5.9	43.2	June 15.8	13.0
2	41.2	136	27	12.2	31.2	58	5.2	42.8	June 17.3	13.3
3	36.7	134	23	5.0	31.3	52	4.7	43.1	June 17.4	14.0
4	34.6	137	24	4.7	29.5	48	4.9	43.5	June 18.4	14.2
5	30.1	137	26	6.2	26.5	43	4.1	43.5	June 19.5	14.0
High ^a	47.9	114	31	18.9	34.7	79	7.3	45.3	June 16.3	11.8
Low ^b	26.6	139	28	7.4	25.7	42	3.3	46.0	June 19.3	14.2
Ceres	41.8	120	30	15.9	32.1	66	7.0	44.3	June 16.7	12.0
Marquis	31.8	125	25	5.1	29.3	48	4.7	44.7	June 19.0	13.7

^a 1658.178.

^b Hope×Ceres, N. N. 1122.

The data in table 3 show at a glance some of the relations which will be expressed later by more refined methods. The heads per row and kernels per spike have no particular relationship to yield, but the contrary is the case with respect to the next four characters in order. No relation is found between height and yield, contrary to what is often found, and with regard to date of heading and percentage of protein a negative relationship exists with yield. The foregoing statements apply generally when one considers the two wheats, high and low, and the two varieties Ceres and Marquis.

ANALYSIS OF VARIANCE

The mean, the standard deviation, and the standard error were calculated for 20 items and are presented in table 4 for both variety and replicate analysis. In the first instance 25 variate means are concerned and in the second instance only 3, as there were 3 replications in the experiment.

TABLE 4.—Statistical constants of wheat from the Fargo regional nursery

No.	Character	Mean	Stand- ard deviation	Standard error				Number ex- cluded ^a
				Variety	Replicate	Significant difference		
						Variety	Replicate	
				Percent	Percent			
1	Yield, mid row.....bushels	37.0	6.6	7.0	2.4	7.7	2.7	16
2	Yield, row B.....do	37.5	6.5	6.2	2.2	7.0	2.4	18
3	Yield per 3 feet, from guard row.....do	37.4	7.0	7.8	2.7	8.8	3.0	15
4	Weight per bushel.....pounds	59.5	2.1	1.1	4	1.9	.7	17
5	Weight per 1,000 kernels.....grams	30.4	3.9	4.2	1.5	4	.1	15
6	Yellow berry.....percent	4.0	5.3	57.1	19.8	6.9	2.4	4
7	Protein.....do	13.7	1.2	4.1	1.4	1.7	.6	9
8	Plants per 3 feet.....number	64.5	12.0	6.1	2.1	12.0	4.0	22
9	Spikes per 3 feet.....do	134.0	18.1	6.5		26.0		10
10	Stooling.....do	2.1	.3	6.0		4		14
11	Fertile spikelets per spike.....number	13.5	1.0	2.6	.9	1.1	.4	20
12	Sterile spikelets per spike.....do	2.3	.5	5.4		4		21
13	Total spikelets per spike.....do	15.8	1.0	2.3	.7	1.1	.3	15
14	Kernels per spike.....do	25.3	2.9	3.9		2.9		20
15	Mid kernels per spike.....do	2.1	.7	16.8		1.1		24
16	Mid kernels per spike.....percent	8.1	4.8	12.1		3.6		23
17	Weight of grain per spike.....decigrams	5.3	1.1	5.9	2.0	9	3	24
18	Date of heading (June).....do	17.7	1.6	2.0		3.0		11
19	Height of plant.....inches	43.3	1.6	1.0	.3	1.3	.5	22
20	Plumpness of kernel, scale 8-1.....do	5.0	1.7	18.7	5.5	2.3	.8	12

^a As outside the limits of significance

When experimental data are given statistical treatment, measures of value are calculated for the various constants. These measures commonly take the form of the "standard error." The standard error calculated for a mean is based upon the squares of the deviations of the variates from their mean. In Fisher's method of analysis of variance (3) the experiment is planned in such a manner that it is possible to remove from the total variability of the experiment all sources of variability other than those due to experimental error. Further, these other sources of variability are analyzed into their components. In the work at hand these components were generally two, one due to varietal differences and one due to replicate differences. Furthermore, in planning such an experiment, the row rows are arranged in a random manner. This arrangement tends to eliminate any association values which might enter if the arrangement were systematic and thus the validity of the standard error is increased. This method was of especial value in this experiment as it made possible the elimination of variability between the replicates, in the calculation of the standard error, which here was of considerable moment. The last column shows the number of varieties which are outside the limits of significance and which could be safely discarded were reliance to be placed upon this one experiment.

The third replication had a lower yield than the first or second. The differences were just significant between replications 2 and 3, but decidedly significant between replications 1 and 3 for all three sets of yields. The lower yield of replication 3 was evidently due to an increased deficiency of soil water along with the associated modification in fertility. The deficient supply of moisture apparently affected in a significant manner nine distinct characters in addition to that of yield. Where replicate differences were not significant,

blanks are indicated. The means of the second replication show changes in the same direction but are insignificant. Within the experiment, then, it is evident that one portion has been subject to a more severe drought effect than has another. Some of the changes involved are indicated in table 5.

TABLE 5.—Constants and other values indicating changes from replication 1 to replication 3

Character	Standard error	Deviation Probable error	Change in replication values	
			Quantity	Percent
Yield per 3 feet.....bushels..	Percent 2.7	4.7	-4.3	-11.1
Plants per 3 feet.....number..	2.1	3.9	+5.0	+8.0
Fertile spikelets per spike.....do..	.9	4.4	-.6	-3.7
Yellow berry.....percent..	10.8	5.0	-4.0	-70.0
Plumpness, 8 classes.....	6.6	5.0	-1.5	-26.4
Protein.....percent..	1.4	5.9	+1.1	+8.3
Bushel weight.....pounds..	.4	5.9	-1.2	-2.0
Height of plant.....inches..	.3	6.2	-.7	-1.6
Weight per 1,000 kernels.....grams..	1.5	7.5	-3.1	-9.7
Weight grain per spike.....centigrams..	2.0	8.9	-8.0	-15.7

The number of plants per 3 feet and the protein content showed increases from the first to the third replication, while the other characters showed decreases. It is not clear why there should be significant differences in stand among the replications, as similar seeding conditions and, apparently, similar soil conditions prevailed at the time of planting. The number of heads per 3 feet also showed increases from replication 1 to 3, but the difference just escaped significance. An initial thicker stand in the third replication might have been carried through to harvest with a lessened yield and with an effect on the other characters of table 4. However, the difference in stand between the two replications corresponds to a difference of but 5.5 pounds, or 7.6 percent, of seed per acre. This difference in rate of seeding would scarcely affect the yield. Recent studies by Hayes, Wilson, and Ausemus (4) show clearly that even larger differences in rate of seeding of wheat do not bring about differences in yield. The correlation between stand and yield is -0.219 ± 0.075 . About one third of the minus products entering into this correlation come from the three Hope $\times$ Reliance wheats. The stand of these wheats is 23 percent above the average while the yield is but 82 percent of the average. The wheats used in seeding were not studied relative to weight of kernel, but it seems probable that the kernels of the Hope $\times$ Reliance wheats weighed less than average. The weight per 1,000 of these three wheats when harvested was but 85 percent of the average. It is questionable if a stand as much as 23 percent above the average would have an injurious effect upon yield. If the greater number of fertile culms showed a too-crowded condition, a compensation would develop in the way of fewer fertile spikelets. Only in pronounced overcrowding could one look for a decreased yield per acre. Evidence on this point is presented by R. W. Smith⁴ who presents results from a date and rate of seeding experiment at Dickinson, N. Dak. An increase in rate of seeding of 20 percent, from 75

⁴ SMITH, R. W. REPORT ON COOPERATIVE CEREAL INVESTIGATIONS AT THE DICKINSON SUBSTATION. 149 p. 1930. (Typewritten report.)

to 90 pounds, resulted in a wholly inappreciable defect in yield of 0.6 bushel, or less than 4 percent, under conditions of drought. Stand counts are not given.

The drier conditions in the third replication showed an effect sufficiently early to reduce the number of fertile spikelets and height of plant. A decrease in plumpness of 1.5 units is associated with an increase in protein of 1.1 percent and a decrease in bushel weight of 1.2 pounds, in a comparison of the first and last replicates. A very marked change came about in the weight of grain per spike, amounting to 9 cg, or 15.7 percent.

CORRELATION DATA

Correlation coefficients were calculated between those characters where causal relationships seemed to be involved. It was particularly desired to learn the various relationships involving yield, and the relationships involving protein content also seemed to be of particular interest. Certain other coefficients were needed in the calculation of partial correlations. Forty-three coefficients are presented in the following tabulation:

Correlation coefficients showing various relationships between yield, protein content, and various other characters

Yield and—		Percent of protein and—	
Weight per bushel	0.438	Percent of yellow berry ..	0.706
Weight per 1,000 kernels ..	.688	Spikes per row	.157
Percent of yellow berry ..	.492	Percent of mid kernels ..	-.292
Percent of protein	-.556	Weight of grain per spike ..	-.586
Plants per row	-.219	Date of heading	.393
Spikes per row	.205	Plumpness	-.615
Stooling	.454	Spikes per row and—	
Fertile spikelets per spike ..	.021	Kernels per spike	-.508
Kernels per spike	.271	Percent of mid kernels per	
Percent of mid kernels per		spike	-.372
spike	.483	Weight of grain per spike ..	-.469
Weight of grain per spike ..	.742	Date of heading	.065
Date of heading	-.571	Plumpness	.021
Height of plant	.063	Percent of mid kernels per	
Plumpness	.587	spike and—	
Weight per bushel and—		Weight of grain per spike ..	.695
Weight per 1,000 kernels ..	.341	Plumpness	.157
Percent of yellow berry ..	.296	Weight of grain per spike and—	
Percent of protein	-.391	Date of heading	-.568
Date of heading	-.178	Number of kernels per	
Plumpness	.664	spike	.584
Weight per 1,000 kernels and—		Plumpness	.521
Percent of yellow berry ..	.466	Plumpness and—	
Percent of protein	-.527	Date of heading	-.340
Spikes per row	-.168	Percent of yellow berry ..	.549
Kernels per spike	.065		
Percent of mid kernels per			
spike	.265		
Weight of grain per spike ..	.722		
Date of heading	-.580		
Plumpness	.630		

The probable errors are not given in the tabulation, but it may be remembered that if the coefficient is as large as ± 0.22 , it is on the threshold of significance; and if the coefficient is as large as ± 0.30 , the ratio of the deviation (from zero) to the probable error is 4, thus indicating marked significance. All but 4 of the 14 coefficients concerned with yield are significant, and most of them are distinctly

so. The highest correlation is weight of grain per spike with yield, which is 0.742. Similar results were obtained with spring wheats by Waldron (10) and by Sprague (9) with winter wheats. Evidently weight of grain per spike is a complex character influenced, for example, by weight of kernel, plumpness, and number of kernels per spike. Partial correlations will lead to a better understanding of these different relationships. One notes a distinctly sharper relationship of yield with mid kernels than with total kernels per spike. This will be discussed later. The correlation of yield with number of heads per row is just below the limit of significance. This is not surprising when one notes the marked negative correlations between heads per row and kernels per spike and also weight of grain per spike.

YIELD AND DELAYED HEADING

The correlation between yield and date of heading is -0.571 . The regression of yield on date of heading is -2.4 bushels for each day's delay in date of heading. The total range in date of heading was 5 days. Bridgeford and Hayes (1) in their studies with spring wheats at Morris, in southern Minnesota, found a positive correlation of 0.27. Other things being equal, one might look for larger yields to accompany a longer photosynthetic period. But conditions may modify this rule, especially with spring-sown cereals.

The history of the introduction of varieties of common wheat into the spring-wheat area shows that those newly introduced wheats which have become successful usually possessed a shorter life history than the wheats which were supplanted, and the comparative yield trials show that these earlier wheats have had the higher yields, in comparison with the older sorts. This empirical fact has not been given a critical analysis. One object in securing earlier varieties, with Marquis for instance, was to obviate early fall frosts in Canadian regions. Marquis, and the still earlier Reward have been considered favorably because of their presumed capacity to escape the attacks of stem rust. The idea that the introduction of early wheats was an advantage because of their capacity to escape conditions of heat and drought, which generally tend to become more intense with the progress of the season, has generally been neglected. It has been shown by Waldron (11) that under conditions of drought, where stem rust has not entered as a disturbing factor, earlier wheats evidently have an advantage in yield.

It is evident, however, that a negative correlation of the sort indicated cannot continue to be linear. The value of an extended photosynthetic period is offset in part by the physical actualities of the season. Evidently there is a golden mean to be secured in a variety whereby maximum yield results will obtain over a series of years, in comparison with either later or earlier wheats. It is believed that the conditions prevailing with respect to the present experiment were fairly typical of an ordinary spring wheat season and this being so, evidently the majority of the wheats were too late in maturing for optimum yield results. It seems fairly evident that one variety may be more efficient in its photosynthetic work than another. In such a case the whole physicochemical complex supporting the photosynthetic activities would need to be well integrated. While it seems rather probable that a situation of this sort holds with Ceres, it is evident that the problem needs to be given critical study.

YIELD AND PROTEIN CONTENT

The correlation of yield to protein content is -0.556 . The regression of yield on protein content is -3.4 bushels, which is the deviation in yield in bushels for each percent of deviation in protein. The total deviation in protein among the varieties is 3.4 percent. The correlation between yield and protein content when date of heading is held constant is reduced from the zero order of -0.556 only to -0.439 . Such a marked relationship as is indicated by this negative correlation is of much economic importance, as a high protein content is desired by the commercial interests while a good yield is naturally a concern of the producer. Where the regression is as great as is indicated here it is evident that a distinct protein premium on the market would be necessary to recompense a farmer for his high-protein wheat. An occasional crop might be high in both yield and protein, but in a series of crops grown under conditions like the foregoing the above regression would probably hold. While it is recognized that the higher percentage of protein is generally in evidence in the drier years, the matter apparently has been given but little statistical treatment, as references in the literature seem to be meager.

The total correlation value of 0.341 between weight per bushel and weight of 1,000 kernels is reduced to -0.133 when plumpness is held constant. As expected, weight per bushel and plumpness are strongly correlated, and it is not surprising that a significant negative correlation, -0.391 , exists between bushel weight and protein content, although this is reduced to a nonsignificant amount when plumpness is held constant. Mangels and Sanderson (7) in a study of miscellaneous samples taken during 4 years, each year being considered individually, found no significant correlation between weight per bushel and protein content. They point out that the lack of correlation they found might be due to the presence of two factors responsible for reduced bushel weight, heat and rust, which act contrarily upon the protein content and that the samples were not sorted with respect to the two differing factors. In the present instance the lower bushel weights, with the exception of one wheat, no. 666, were due to heat and drought injury. This one wheat contributed but little to the product moment.

The correlation of weight per 1,000 kernels to percentage of yellow berry, 0.466, is reduced to a nonsignificant amount when plumpness is held constant. Likewise the coefficient of -0.527 concerned with protein content and weight per 1,000 kernels is reduced to the horizon of significance when plumpness is held constant. The relationship of kernel weight to date of heading shows a negative correlation, -0.580 , and this is reduced to only -0.499 when plumpness is held constant. In this experiment the later heading wheats have the smaller kernels irrespective of plumpness. One would scarcely expect this rule to hold generally, especially where optimum conditions are afforded for maturing the grain and when adaptable varieties are grown.

PROTEIN CONTENT

Seven of the nine correlations involving protein content are minus and significant. In only one relationship, where date of heading was concerned, was the correlation positive and significant. With these

wheats, at least, in a season where conditions favor quite fair yields and in the face of rather adverse heat and moisture conditions and with rust injury of little moment, high protein content is gained at the expense of other characters, some of them of marked desirability, such as yield and bushel weight. One notes a high negative correlation, -0.706 , between the percentage of protein and the percentage of yellow berry. The presence of yellow berry is one of the indicators of low grade; a wheat must have 85 percent of dark vitreous kernels in order to grade No. 1 Hard.

Mangels (6) in a study of numerous milling samples during 4 years, 1922 to 1925, found that the relationship between protein content and percentage of dark kernels varied considerably from year to year and that correlation might be very low. As he worked with a complex of samples, including many which evidently had been affected in starchiness and bushel weight by shriveling due to the rust organism (Mangels and Sanderson (?)), and presumably with samples affected by weathering, it is evident that his coefficients might not be comparable to the one given here, where the problem has been rendered more simple by restricting the study to samples of which the protein content and starchiness were conditioned by varietal and growing conditions, and which were not affected appreciably by disease.

In the present case the regression of protein content on percentage of yellow berry is -0.16 . Thus a 10 percent deviation in yellow berry would be associated, generally, with about a 1.5 percent negative deviation in protein content. This appears to be a rather sharp reaction. The percentage of yellow berry per variety varied from 0 to 18.7. The third replicate of the experiment, as shown in table 5, had a significantly lower yield and a lower percentage of yellow berry, and a sharp increase in protein content. These differences are all very significant.

A negative correlation of -0.469 exists between spikes per row and weight of grain per spike. A similar correlation of -0.72 was found by Bridgford and Hayes (1). In the present instance the regression is such that a deviation of 10 percent from the mean in number of heads leads to a minus deviation from the mean in weight of grain per head of 7.4 percent.

The character of percentage of mid kernels per spike is of particular interest as some of the higher yielding wheats, including Ceres and its two allies, are characterized by a high percentage of mid kernels. This character is evidently derived from the Kota parent as indicated by Waldron (11). The correlation between weight of grain per spike and percentage of mid kernels is 0.695, and the regression of grain weight per spike on percentage of mid kernels is 1.6. Thus a deviation of mid kernel percent of three units results in a deviation of 9 percent of weight of grain per head. The grain weight per head, as was pointed out, is strongly correlated with yield.

PARTIAL CORRELATIONS

As yield is probably the most important character of the experiment, partial correlations were calculated to show the relationship of yield to different characters when one or more of the other characters were held constant. The characters considered, besides (1) yield, are (2) spikes per row, (3) percentage of mid kernels, (4) weight per

1,000 kernels, (5) plumpness, and (6) weight of grain per spike. The total correlations, and all the partial correlations where yield is considered as a variable, are given in table 6. The numbers used in the entries in the table refer to the numbered characters just given.

TABLE 6.—Total and partial correlations involving yield as the variable in all cases*

Yield (1) correlated with -									
Spikes per row (2)		Percentage of mid kernels (3)		Weight per 1,000 kernels (4)		Plumpness (5)		Weight of grain per spike (6)	
	Correlation		Correlation		Correlation		Correlation		Correlation
12	0.205	13	0.483	14	0.688	15	0.587	16	0.742
12 3	.473	13 2	.615	14 2	.748	15 2	.595	16 2	.969
12 4	.447	13. 4	.430	14 3	.663	15 3	.591	16 3	.645
12 5	.258	13 5	.489	14 5	.505	15 4	.272	16 4	.488
12 6	.933	13. 6	-.068	14 6	.328	15 6	.351	16 5	.631
12 34	.702	13. 24	.695	14 23	.796	15 23	.626	16 23	.950
12 35	.524	13. 25	.644	14 25	.594	15. 24	.225	16. 24	.931
12 36	.932	13 26	-.002	14 26	.194	15 26	.066	16 25	.952
12 45	.424	13. 45	.450	14. 35	.469	15 34	.308	16 34	.276
12 46	.927	13 46	.107	14 36	.337	15 36	.349	16 35	.460
12 56	.923	13 56	.056	14. 56	.209	15. 46	.246	16 45	.476
12 345	.693	13 245	.703	14 235	.664	15 234	.267	16 234	.863
12 346	.927	13 246	.105	14 236	.220	15 236	.099	16 235	.916
12 356	.923	13 256	.021	14 256	.183	15 246	-.006	16 245	.927
12 456	.922	13 456	.153	14. 356	.252	15 346	.268	16 345	.230
12 3456	.921	13 2456	.106	14. 2356	.210	15 2346	.014	16 2345	.851

* The statements concerned with the significance of the coefficients of correlation following the tabulation on page 135 applies likewise to the coefficients of table 6

The partial correlation coefficients were calculated from 75 variates. This is probably too small a number to give the most satisfactory results, but the individual values for the most part are not single observations. The yields are correlated fairly well with the yields obtained from the two other rows of the replicates. The regression of the 3-foot yields on the rod row yields is 0.7 bushel. The errors in yield can be assigned to both sets of rows; whether the larger measure of errors belong to the 3-foot rows cannot be determined. The standard deviation of the 3-foot rows is 6.6 percent greater than that for the rod rows, and the difference in the two standard deviations is 0.43 ± 0.53 .

The variates of some of the other characters are based on means. The characters with respect to the spike were taken on a sample of 50 heads, or nearly that number. Such a character as plumpness would likely not be much modified if additional readings had been made. The marked changes which result when this or that variable is held constant make it evident that the partial correlations are of real significance. For instance, the total correlation of yield and spikes per row is 0.205; but if the weight of grain per spike is constant, then the partial correlation has the high value of 0.933. It is evident that with respect to yield the two components, number of spikes per row and weight of grain per spike, form nearly or quite a closed system. The difference between 1 and the partial correlation value of 0.933 would be the sampling error. A study of table 6 shows how the presence or absence of a character held constant changes the value of a coefficient.

MULTIPLE CORRELATION

In interpreting the partial correlations of table 6 it is important to consider multiple correlation. A multiple-correlation coefficient can be calculated from partial coefficients, according to Yule (12, p. 248) by the following formula:

$$1 - R^2_{1 \ 23 \dots n} = (1 - r^2_{12}) (1 - r^2_{13 \ 2}) (1 - r^2_{14 \ 23}) \dots (1 - r^2_{1n \ 23 \dots (n-1)}).$$

The value of R^2 , termed by Ezekiel (2) the coefficient of multiple determination, is the value most commonly used, rather than R , and expresses the proportion of the variance of the dependent variable explained by the independent variables under consideration. We may consider the partial correlation $r_{16 \ 24}$. By using the above formula the two values may be calculated:

$$R^2_{1 \ 24} = 0.578;$$

$$R^2_{1 \ 216} = 0.944.$$

When spikes per row (2) and weight per 1,000 kernels (4) alone are considered, it is apparent that 57.8 percent of the variance in yield is explained and that 42.2 percent remains unexplained. Similarly when weight of grain per head (6) is made a part of the calculation as a third variable, it is found that 94.4 percent of the variance of yield is explained by a consideration of the three variables while but 5.6 percent remains unexplained. It is evident that the addition of the third independent variable increased the explained variance by 36.6 percent. This value may be expressed in relation to the 42.2 percent left unexplained when only the two factors are considered, by dividing 36.6 by 42.2. The quotient, expressed as 86.7 percent, is the additional explanation, expressed on the basis of the original unexplained total of 42.2 percent, brought about by the introduction of the third independent variable. The square root of 0.867, 0.931, is the partial correlation of $r_{16 \ 24}$. The partial correlation coefficient is thus defined in a somewhat different manner.

In studying the correlation of yield and heads per row, one notes the total correlation, 0.205, is comparatively low, but that the value immediately rises when the percentage of mid kernels, the weight per 1,000 kernels, or the weight of grain per head is held constant. The value is exceptionally high in the last instance, the coefficient being 0.933. The effect on the total correlation is inappreciable when plumpness is held constant, or when plumpness is introduced as a constant in addition to other characters held constant. It seems evident from a comparison of the two coefficients, $r_{12 \ 6} = 0.933$, and $r_{12 \ 3456} = 0.921$, that the factor, weight of grain per head, might be considered a composite of the characters of percentage of mid kernels, weight of 1,000 kernels, and plumpness. Thus if the character, weight of grain per head, is considered, then the three other characters become automatically considered. This becomes apparent in some of the other columns. The total correlation of yield and percentage of mid kernels is 0.483, but when weight of grain per head is held constant the correlation becomes essentially zero. A corresponding decrease in values is observed when the total correlations of yield and weight per 1,000 kernels and yield and plumpness are considered, in comparison with the same correlations when weight of grain per head is held constant.

The coefficient of multiple determination was calculated and found to be $R^2_{1\ 23456} = 0.944$. This, or 94.4 percent, indicates the proportionate amount of variance in yield, the dependent variable, which was brought about by the five independent variables indicated. It follows that only 5.6 percent of the squared variability of yield remains unexplained after the five factors indicated are taken into consideration.

If the relationship of weight of grain per spike to the three characters, percentage of mid kernels, weight per 1,000 kernels, and plumpness are considered, when weight of grain per spike is used as the dependent variable, $R^2_{6\ 345} = 80.3$ percent. Evidently in this case considerably the larger portion of the variance of weight of grain per spike is due to these three variables. The matter can be considered from another angle by omitting this character as such and considering its three components and the number of spikes per row.

PARTIAL REGRESSION COEFFICIENTS

The coefficients of partial or net regression were calculated resulting in the equation

$$\begin{aligned} \text{Yield} & -0.183 \text{ spikes per row} \\ & + .713 \text{ percent of mid kernels} \\ & + .978 \text{ weight per 1,000 kernels} \\ & + .628 \text{ plumpness} \quad 25.83 \text{ bushels} \end{aligned}$$

The regression or *b* values of the foregoing equation are all significant; the first three very decidedly so. If this equation is used and the appropriate means of table 4 inserted, the calculated yield will equal the mean yield of 37.4. If one uses average values in this equation for some of the characters and a varietal value for some other character the result is of interest for some of the varieties. If the percentage of mid kernels of Ceres (table 3) is inserted instead of the corresponding average value the resultant yield is 42.9 bushels, which is 1.1 bushels higher than the Ceres yield and 0.4 bushel below its calculated yield. By using the weight per 1,000 kernels for Ceres the resulting yield is 39.0 bushels, which is 2.8 bushels below the yield of Ceres. One might conclude from this that the Ceres character of high percentage of mid kernels was more responsible than any of the other characters considered for the rather high yield of this variety. The same statement would hold true for 1658.178, a sib of Ceres. If these same calculations are made for Marquis by inserting first the Marquis percentage of mid kernels and then the Marquis weight per 1,000 kernels, the calculated yield in the first instance is 35.2 bushels, which is 2.2 bushels below the general average, and 3.4 bushels above the Marquis yield. Similarly by using the weight per 1,000 kernels of Marquis the calculated yield comes to 36.3 bushels. Thus the use of weight per 1,000 kernels reduces the yield less from the average than does the use of percentage of mid kernels.

The coefficient of multiple determination, involving the relationship of the four characters given above to yield, is

$$R^2_{1\ 2345} = 0.783.$$

This coefficient shows the combined importance of the four independent characters in measuring the variance of yield, the dependent

factor. It is evident that 21.7 percent of the variance remains unexplained.

In measuring the relative importance of the different coefficients entering into the equation of partial regression already given, three sets of values are given. The first set consists of the partial correlation squared. A squared correlation coefficient, as previously indicated, is a measure of the importance of the increased variance which can be explained by the consideration of an added variable. The second set consists of the beta coefficients, commonly calculated, described by Ezekiel (2, p. 183); these are calculated by the formula

$$\beta_{12.34} = b_{12.34} \frac{\sigma_2}{\sigma_1}$$

This formula transforms the partial-regression coefficient as expressed in actual values into coefficients expressed in terms of the standard deviations involved. It is thus rendered more comparable. It is possible to break up the coefficient of multiple determination into components which will add up to its total value. These components are called coefficients of separate determination and constitute the third set. The limitations of these coefficients are indicated by Ezekiel (2, p. 381). The three sets of values are given in table 7.

TABLE 7.—Various coefficients indicating how the regression coefficient values may be measured with respect to (2) spikes per row, (3) percentage mid kernels, (4) weight per 1,000 kernels, and (5) plumpness as affecting (1) yield

Designation	Coefficients		
	Partial correlation (squared)	Beta	Separate determination
12.345.....	0.480	0.474	0.096
13.245.....	.494	.493	.234
14.235.....	.441	.538	.365
15.234.....	.071	.153	.088
Total.....			.783

The value 0.096 is much below 0.480 and 0.474, and this is evidently due to the low total correlation between yield and heads per row. There is no positive correlation between heads per row and the other independent variables. The low value when plumpness is the independent variable is not due to the low total correlation with yield but rather to the correlation of plumpness with weight per 1,000 kernels, which is 0.630.

Evidently weight per 1,000 kernels may be of somewhat more consequence in affecting yield than is percentage of mid kernels. That this is not true in all cases was shown with Ceres and 1658.178. The effect of spikes per row upon yield is probably somewhat exaggerated, as indicated in the first two columns where values are 0.480 and 0.474. The negative correlation between heads per row and weight of grain per head is -0.469 , and this fact seems to give undue influence to heads per row when the other yield characters are held constant. One suspects that the coefficient of separate determination

may indicate the relative weightings to be assigned to the four characters in their effect upon yield somewhat better than do either of the other two sets.

DISCUSSION

The present work seems to afford a good opportunity to study the effects of drought and heat on wheat yields largely uninfluenced by disease. The drought and heat injuries were fortunately not extreme, and this fact, in connection with a rather marked divergence among the varieties relative to yield, has added further to the value of the experiment. Some of the physical characters studied are evidently quite highly associated with yield as judged by coefficients of correlation. The weight of grain per spike has a marked correlation with yield, equal to 0.969, when spikes per row, or stand, is held constant. An analysis of this character shows that it is made up mainly of the three components, percentage of mid kernels, weight per 1,000 kernels, and plumpness. Comparative plumpness in this experiment was modified mainly by heat and moisture conditions. Differences in plumpness were not truly inherent in the varieties but were the result of environment. It is presumed that the kernels of all varieties would have been of about the same degree of plumpness had optimum moisture and heat conditions prevailed. Weight per 1,000 kernels is obviously affected by plumpness, but besides this there are inherent varietal differences. Apparently yield in this experiment is affected by differences in kernel weight. The total correlation between yield and kernel weight is 0.688, and when plumpness is held constant the correlation is still 0.505, a very significant figure. The total correlation between weight of grain per spike and weight of 1,000 kernels is 0.722, and when plumpness is held constant the coefficient is 0.594. The character, percentage of mid kernels, is affected probably less by the prevailing heat and drought than either of the characters just considered. The total correlation between yield and percentage of mid kernels is 0.483, and this coefficient is not changed appreciably when plumpness is held constant. A confirmation of the foregoing is seen in a comparison of the replicate means. The means for plumpness and weight per 1,000 kernels of the third replication are much smaller, judged by probable errors, than the corresponding means of the first replication (table 5).

Considering the percentage of mid kernels, the difference between the two replicate means is wholly inappreciable. It was shown that number of spikes per row and weight of grain per spike are responsible for 94.1 percent of the variance of yield. Considering only weight of grain per spike as affecting yield the coefficient of determination is equal to 55 percent, or 55 percent of the variance, or squared variability, of yield is due to weight of grain per spike. The weight of grain per spike was analyzed into the three components already discussed. The coefficient of multiple determination, indicating the proportion of variance of weight of grain per spike brought about by the three characters, percentage mid kernels, weight per 1,000 kernels, and plumpness, was found to be $R^2=80.3$ percent. This is appreciably less than the value of 94.1 percent given previously, which is the proportional variance of yield explained through the medium of the two factors, spikes per row and weight of grain per spike. It was thought that the number of kernels per spike might

contribute something to the variance of weight of grain per spike. A recalculation of the value of R^2 with these 4 contributing factors, instead of the 3 just given, raises the value of R^2 from 80.3 to 85.0 percent. Thus there still remains an appreciable amount of the variance of weight of grain per spike unaccounted for even though the various contributing factors seem to have been included in the coefficient. The coefficients of determination, the squared values of r of table 6, for these four contributing factors are as follows:

Variance of grain weight per spike brought about by--

(1) Percent of mid kernels	0.484
(2) Weight per 1,000 kernels	.521
(3) Plumpness	.271
(4) Number of kernels per spike	.341

While the values as given indicate somewhat the relative influence of the 4 factors the comparative values of 3 of these factors, omitting the fourth, are better shown in table 7. It is evident that both percentage of mid kernels and weight per 1,000 kernels are relatively important factors in influencing yield in this experiment.

PROTEIN CONTENT

A negative correlation of -0.556 was shown between yield and protein content. So little work has been done in this field that it is difficult to say whether this particular case is unusual or not. It is known that the protein content of wheat varies greatly with respect to the available nitrogen in the soil, and protein content may be affected more decidedly than yield, as evidenced by this negative correlation. In the present instance the soil may have been lacking in available nitrogen or it may be that certain of the varieties stood in such a relationship to the seasonal conditions that the soil nitrogen was not of first importance in bringing about the higher yields. If available soil nitrogen tended to be a limiting factor in this experiment, it would be desirable to learn whether the lower yields of some of the varieties were conditioned by the deficient nitrogen. Stated in another way, certain of the better yielding wheats might be able to carry on active photosynthesis in the face of a limitation of nitrogen. The later and lower yielding wheats had the higher content of protein. The high protein content of these varieties did not compensate for the low yield in the total amount of protein per acre. If the 6 high-yielding and the 6 low-yielding varieties are considered, the difference in amount of protein per acre, in favor of the high-yielding sorts, is 85 ± 8.5 pounds of protein per acre. The shortness of growing period was evidently only one factor in bringing about the low protein content. When the date of heading is held constant, the correlation between yield and protein content is still -0.439 . However, the correlation between the date of heading and the date harvested has only the moderate value of 0.358 . The date wheat is fully ripe is more difficult to determine than the date of heading.

Positive correlations have been shown to exist between yield and spikes per row and yield and weight of grain per head. The last-mentioned character has been analyzed into other characters. With a rather strong negative correlation existing between yield and protein content, negative coefficients may be looked for between protein content and those characters contributing to yield. Evidently in the

relationship between protein and percentage of mid kernels, for example, yield is an influencing factor. The correlation values between protein content and yield factors, both total and partial when yield is held constant, are shown in table 9.

TABLE 9.—*Correlation of protein content with the yield factors indicated*^a

Character	Total	Partial correlations with yield held constant
Number of spikes per row	0.157	0.333
Weight of grain per spike	-.586	-.312
Percentage of mid kernels	-.202	-.032
Weight per 1,000 kernels	-.527	-.240
Plumpness	-.615	-.429

^a Correlation values greater than ± 0.22 are of significance

The total correlation of yield with spikes per row was only 0.205. The correlation of protein with this character is positive, the value being 0.157, and this value becomes significant when yield is held constant. In all the other cases shown in table 9 the correlation values are negative, both total and partial. The lowest values are percentage of mid kernels, and when yield is held constant the correlation value is essentially 0. In considering weight per 1,000 kernels the correlation with flour protein might have shown a smaller numerical value, for with smaller kernels the percentage of aleurone layer increases. But plumpness enters into the relationship, and this factor may be responsible in part for the negative correlation between weight of kernels and protein. In fact when both yield and plumpness are held constant, the correlation between protein content and weight per 1,000 kernels falls to 0.090. Attention has been called to the negative correlation between yield and protein content. One would look for a decided lessening of this correlation value if yield factors were held constant. The correlation between yield and protein content when spikes per row and weight of grain per head are held constant is -0.195 . This value is slightly below the horizon of significance and shows a marked drop from the total correlation of -0.556 .

SUMMARY

Twenty-five hard red spring wheats were grown in randomized rod rows at Fargo, N.Dak., during the season of 1932 under conditions of high temperature and low precipitation, and under a light incidence of disease.

The wide range of yields, from 24 to 46 bushels per acre, was due in large measure to conditions of drought and heat. One replication, because of less available moisture, showed a significantly lower yield than did the others, and significant replicate differences were found for nine other characters.

Significant total correlation coefficients were found between yield and 11 of the 14 characters where calculations were made. The largest coefficient, 0.742, involved weight of grain per spike. The two outstanding negative correlations, with yield, are -0.571 , where date of heading is involved, and -0.556 , with respect to protein

content. Evidently a lengthened period of photosynthetic activity, normally considered desirable for good yields, may be more than offset by injurious end seasonal conditions of low moisture and high temperature. The supplanting of the older, longer period wheat varieties by the newer, shorter period varieties in the spring-wheat area is a practical confirmation of the present findings.

The correlation of yield to spikes per row is 0.205 and to weight of grain per spike is 0.742. The coefficient of multiple determination for these two factors with respect to yield is $R^2_{1,26} = 94.1$ percent. Thus only 5.9 percent of the variance of yield remains unaccounted for when these two characters are considered. The correlation of yield to weight of grain per spike, when the number of spikes per row is held constant, reaches a high value of 0.967. This is the maximum value in this relationship, despite the holding constant of additional characters. Evidently the factors of number of spikes per row and weight of grain per spike, as affecting yield, constitute a nearly or quite closed system.

The character, weight of grain per spike, is analyzed into the three characters percentage of mid kernels, weight per 1,000 kernels, and plumpness. The three individual correlations are: Mid kernels, 0.695; weight per 1,000 kernels, 0.722; and plumpness, 0.521. The coefficient of multiple determination of weight of grain per spike with respect to these three characters is $R^2_{6,345} = 80.3$ percent. Here 19.7 percent of the variance of weight of grain per spike remains unaccounted for.

When yield is analyzed into 4 factors, the 3 just considered and number of spikes per row, the coefficient of multiple determination with respect to yield is $R^2_{1,2345} = 78.3$ percent, leaving 21.7 percent of yield variance not accounted for. If each of the 4 contributing factors is dropped in succession and comparisons are made, it is found that the addition of percentage of mid kernels results in a greater additional explanation of yield variance than any of the other 4 characters, whereas the character plumpness is of least moment in this respect. Other comparisons indicate that weight per 1,000 kernels is of greater moment than the percentage of mid kernels.

The partial regression coefficients of yield on the 4 characters, spikes per row, percentage of mid kernels, weight per 1,000 kernels, and plumpness are all significant, the first 3 very decidedly so. If the regression equation is used with average values except that values of Ceres and its allies are substituted for percentage of mid kernels or weight per 1,000 kernels, the results indicate that the former character contributes more than the latter to yields approximating those of Ceres. A similar calculation involving Marquis indicates that the use of the factor percentage of mid kernels carries the calculated yield farther from the general average and nearer to the actual Marquis yield than happens when the factor of weight per 1,000 kernels is used.

Of the 9 correlation coefficients calculated involving protein content, 7 are minus and significant. Under conditions similar to those in the present experiment, high protein content is secured at the expense of other desirable characters, such as yield and weight per bushel. In only one instance, which involved the date of heading, was the correlation coefficient with protein both positive and significant.

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RUBBER CONTENT OF VARIOUS SPECIES OF GOLDENROD²

By LOREN G. POLHAMUS²

Associate technologist, Division of Cotton, Rubber, and Other Tropical Plants,
Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The native plants in which rubber has been found in sufficient quantity to justify intensive study are species of *Asclepias*, *Chrysanthamnus*, *Parthenium*, and *Solidago*. The field of possible rubber-bearing plants has been only partially explored, and few species have been collected at all seasons of the year and under sufficiently diverse conditions of growth to determine the possible range of rubber content. Little is known as to the effect of cultivation on the rubber content of plants, the present indications being that some plants produce rubber under conditions of active growth, while others produce rubber only when active growth is retarded.

Since the announcement of the late Thomas A. Edison's discovery that goldenrod contains rubber, the goldenrods (*Solidago*) have attracted much attention. Nearly 100 species of *Solidago* have been recognized by botanists in the United States. Some of these are confined to local areas; others are widely distributed and show many variations in different parts of their range. Just as there is a great difference among the species in flower, leaf, and growth characteristics, there is also a great difference in rubber content. Several of the species have shown considerable variation in the rubber content of samples collected in different localities.

MATERIAL AND METHODS

The plants used for analysis were collected in 1931 at several localities near Washington, D.C., in the vicinity of Charleston, S.C., and in the northern part of Florida. Whenever sufficient material was available separate analyses were made of the leaves, stems, and roots. In order to obtain information as to the value of the entire plant as it might be harvested with a mowing machine, several plants of each lot with all leaves and stems were made into a composite sample.

In general the method described by Hall and Goodspeed³ was followed in making the analyses. According to this method the finely ground sample is first extracted with acetone to remove all

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² The plants used for the analyses were collected by Mildred Pladeck, of the Division of Seed Investigations, Bureau of Plant Industry, and W. H. Jenkins, of the Division of Cotton, Rubber, and Other Tropical Plants. Botanical identifications were made by Miss Pladeck and S. F. Blake, of the Division of Botany.

³ HALL, H. M., and GOODSPEED, T. H. A RUBBER PLANT SURVEY OF WESTERN NORTH AMERICA. Calif. Univ. Pubs., Bot. 7 [159]-278, illus, 1919.

resins and fats, and then with benzol to remove the rubber. The process as described by Hall and Goodspeed was modified in that a 25-ml Gooch filtering crucible was used to replace the siphon cup in the Bailey-Walker extraction apparatus and the extraction period was lengthened to 6 hours instead of 3 for each extraction. The 5-g samples were weighed accurately to 0.002 g, and the rubber and resin extracts were weighed to 0.0001 g. The samples were analyzed in duplicate, the agreement between duplicate samples being sufficiently close to consider the rubber percentages accurate to 0.10 percent.

RESULTS OF ANALYSES

RUBBER CONFINED TO LEAVES

While it was impossible to have separate stem and root samples of every lot, in no case was a significant percentage of rubber found in the stems or roots of any plant, and the results of this series of analyses indicate that none of the species examined can be considered of any importance except on the basis of the rubber in the leaves. Since in goldenrod the leaves represent a very small proportion of the plant, only species having a high rubber content in the leaves, together with a large leaf production, would be considered important. Species such as *Solidago minor* and *S. tenuifolia*, which have minute slender leaves and a comparatively large amount of stem, are unimportant even though they rank high on the basis of the rubber content of the leaves. *S. rugosa*, which produces a comparatively large amount of leaves, ran consistently high on the basis of the rubber content of entire plants, although the highest rubber content found was only 1.55 percent.

RUBBER CONTENT OF LEAVES OF DIFFERENT SPECIES OF GOLDENROD

Leaf specimens from 24 species of goldenrod were tested. The rubber content of the leaves of each species in percentage of the total dry weight of the sample is shown in table 1. The number of specimens analyzed, the high and the low percentage if more than one specimen was analyzed, and the mean if three or more were analyzed, are also shown for each species. While a great variation in rubber content was found in the species of which several samples were collected, in general it was possible to grade them on the basis of the rubber in the leaves into species with high and species with low rubber content. Of the 24 species, a sample of *Solidago altissima* had the highest rubber content, 6.34 percent. Samples of 10 other species had a rubber content of more than 3 percent. The lowest percentage was found in *S. squarrosa*, with a rubber content of 0.56, but in both *S. erecta* and *S. racemosa* the rubber content was less than 1 percent.

A high variation in the rubber content of the leaves was found in some high-yielding species, due either to individual plant variation or to habitat or seasonal effect. This variation is sufficient to indicate a real possibility of plant selection on the basis of rubber content and the development of cultural practices based on optimum conditions of rubber production. That some of the species were found to be more variable than others may be due to the fact that some species, being more common than others, were collected under a greater variety of conditions; or it may be that some species are less well established or have been crossed with other species.

TABLE 1.—Rubber content (percentage of dry weight) of the leaves of various species of *Solidago*

Species	Analyses	Rubber content		
		High	Low	Mean
	Number	Percent	Percent	Percent
<i>Solidago altissima</i> L.	12	6.34	1.38	3.45
<i>S. arguta</i> Ait.	1	1.02	—	—
<i>S. bicolor</i> L.	3	1.20	.89	1.03
<i>S. carnea</i> L.	1	2.46	—	—
<i>S. erecta</i> Pursh.	1	.80	—	—
<i>S. fistulosa</i> Mill.	4	4.48	2.08	2.99
<i>S. flexicaulis</i> L.	2	3.46	1.47	—
<i>S. graminifolia</i> (L.) Salisb.	2	1.07	1.03	—
<i>S. juncea</i> Ait.	2	1.38	.93	—
<i>S. minor</i> (Michx.) Fernald.	5	3.53	2.63	3.00
<i>S. neglecta</i> Torr. and Gray.	1	3.36	—	—
<i>S. nemoralis</i> Ait.	2	2.43	2.38	—
<i>S. puberula</i> Nutt.	1	2.35	1.20	1.62
<i>S. racemosa</i> Greene.	1	.92	—	—
<i>S. rigida</i> L.	1	3.10	—	—
<i>S. rugosa</i> Mill.	7	3.51	2.63	2.95
<i>S. sempervirens</i> L.	3	3.91	2.22	3.02
<i>S. serotina</i> Ait.	5	5.64	2.40	3.92
<i>S. speciosa</i> Nutt.	2	3.12	2.50	—
<i>S. squarrosa</i> Muhl.	1	.56	—	—
<i>S. stricta</i> Ait.	1	2.87	—	—
<i>S. suarcorensis</i> Schoepf (<i>S. odora</i> Ait.).	3	3.31	2.26	2.68
<i>S. tenuifolia</i> Pursh.	1	2.91	—	—
<i>S. ulmifolia</i> Muhl.	2	1.84	1.28	—

The variation is particularly striking in the case of *Solidago altissima*, a very common species collected from 14 localities. Leaves of 12 of the 14 samples collected were analyzed, the lowest percentage of rubber being 1.38, the highest 6.34, and the mean of the 12 samples 3.45. *S. serotina*, of which 5 samples were analyzed, also showed considerable variation in rubber content, the range being from 2.40 to 5.64 percent. Of the species having leaves with a relatively high rubber content, *S. minor* and *S. rugosa* showed the least variation.

In general, the species grouped together by taxonomists were found to be grouped on the basis of comparative rubber content. A conspicuous deviation from this rule, however, was found in three species, *Solidago graminifolia*, *S. minor*, and *S. tenuifolia*, which usually are classified under the subgenus *euthamia*. *S. graminifolia*, with percentages of 1.03 and 1.07, had a low rubber content, while *S. minor* with a high content of 3.53 percent and *S. tenuifolia* with 2.91 percent were relatively high in rubber.

RUBBER CONTENT OF GOLDENROD AS COMPARED WITH THAT OF OTHER PLANTS

The rubber content of goldenrod compares favorably with that of other native plants. The highest rubber content found in the present investigation was 6.34 percent, but a rubber content as high as 8 percent was found in Edison's experiments.⁴ Guayule has been reported to produce as high as 15 percent, but requires a period of 4 years to attain this content.

Hall and Goodspeed⁵ found an old sample of rabbitbrush (*Chrysothamnus nauseosus*) with a rubber content of 6.57 percent. These investigators also found an old gnarled plant of *Aplopappus nanus* from near Benton, Calif., with a rubber content of 9.46 percent.

⁴ Oral communication.

⁵ HALL, H. M., and GOODSPEED, T. H. Op. cit.

Of the native rubber-bearing plants producing rubber in a single season, the highest percentages of rubber have been found in one of the desert milkweeds, *Asclepias subulata*. Hall and Long⁶ reported as high as 6.5 percent rubber in young stems. These investigators also found 5.1 percent rubber in one sample of hemp dogbane (*Apocynum cannabinum*). Specimens of other genera have been reported to contain up to 4 percent of rubber.

NONRUBBER CONSTITUENTS IN GOLDENROD

In goldenrod there is, in addition to the rubber, an appreciable quantity of nonrubber substance which can be extracted with acetone. This material consists primarily of resins, although there are also small amounts of fats, oils, and sugars. Little is known of the chemical identity of the resins or of the possible use to which they might be put, although it has been suggested in the case of similar resins from other rubber-bearing plants that they might be used in paint or varnish or in the manufacture of soap. Since significant amounts have been found in several species of goldenrod, the by-product value of the resins is worthy of consideration.

The highest acetone extract found was 26.45 percent in 1 leaf sample of *Solidago minor*. The mean of the 5 samples of this species was 21.03 percent, and the lowest percentage was 16.24. Acetone extracts greater than 15 percent also were obtained from samples of 4 other species. These included 3 of the 4 samples of *S. fistulosa*, 2 out of 4 samples of *S. puberula*, 1 out of 5 samples of *S. serotina*, and the single sample of *S. neglecta* analyzed.

A positive but very small correlation of 0.397 ± 0.070 was found between the rubber content and the resin content of the 68 leaf samples of *Solidago*. It would appear probable, therefore, that in *Solidago* there is a relation between the rubber content and the acetone extract, but further study under controlled conditions will be necessary to determine adequately the extent of this relation.

SUMMARY

Samples of 24 species of goldenrod (*Solidago*) were analyzed, and significant quantities of rubber were found in the leaves of some of the samples. None of the species contained more than a trace of rubber in any portion of the plant except the leaves. The highest rubber content found was 6.34 percent in one sample of *Solidago altissima*. A rubber content greater than 3.50 percent was found also in specimens of *S. fistulosa*, *S. minor*, *S. rugosa*, *S. sempervirens*, and *S. serotina*. These percentages justify the investigation of selection possibilities in relation to rubber content and the development of cultural practices conducive to the formation of rubber.

⁶HALL, H. M., and LONG, F. L. RUBBER CONTENT OF NORTH AMERICAN PLANTS. 65 p., illus. 1931. (Carnegie Inst. Wash., Pub. 313.) Washington, D.C.

INJURY TO PEARS CAUSED BY PAPER LINERS IMPREGNATED WITH SODIUM SILICATE¹

By D. H. ROSE, *senior physiologist*, and J. M. LUTZ, *junior physiologist*, *Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Pears of all varieties have one or more layers of stone cells just under the skin, and because of the mechanical resistance offered by these cells the pears are very susceptible to damage by rubbing or bruising. Moreover, the skin of pears usually turns dark brown or black after an injury; hence, after the treatment ordinarily given to pears during the picking and packing processes they are likely to show some discoloration when they reach the market. For the last several years, however, a brown spotting has been observed on certain varieties of pears, both in carload shipments just arrived on the market and in cold-storage lots, which is different in some respects from that caused by rubbing or bruising. It is found on fruits that have been in contact with the corrugated paper used for lining the boxes, or with the excelsior-filled pads used in pear boxes to prevent bruising of the fruit. The spotting is brown in color and usually only superficial. In many instances, especially with the Bosc pear, it occurs in bands crosswise of the fruit, corresponding to the corrugations of the paper liner (fig. 1, A). Often, however, it occurs only as an irregular-shaped brown spot one fourth to 1 inch in diameter. The spotting is sometimes found on all the fruits in a box that touch the corrugated paper and may thus involve one third or more of the contents of a box. It has not been seen on pears or the portions of pears that have not been in contact with a corrugated liner or an excelsior-filled pad.

The injury has been reported several times by inspectors of the food products inspection service of the Bureau of Agricultural Economics, United States Department of Agriculture, usually on pears of the Winter Nelis, Bosc, and P. Barry varieties, all of which show more or less russetting of the skin. It has never been found on smooth-skinned pears, although a dark discoloration on pears of the Anjou variety, the result of bruising or rubbing against the paper liner or against other pears, has sometimes been confused with it. A similar injury caused by bruising or rubbing is frequently found on pears of the Bosc variety.

PRELIMINARY EXPERIMENTS

Analyses made in 1926 by T. D. Jarrell, of the Bureau of Chemistry and Soils, United States Department of Agriculture, from pieces of liner material that had been in contact with affected pears, showed the presence of about 1 percent of sodium silicate. A solution obtained by water extraction of pieces of the liner had a definite alkaline reaction. It seemed probable, therefore, that the spotting was caused by the sodium silicate, which had been used as a binder to

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hold the two parts of the corrugated paper together. In order to test this, small pieces of absorbent cotton were dipped in one fourth, one half, and 1 percent solutions of sodium silicate; most of the solution was squeezed out, and the cotton was then laid on unspotted portions of affected Winter Nelis pears. After the fruit had been held in a moist chamber for 24 hours, spots like those originally noted on the pears were found under all the cotton that had been moistened with a silicate solution. Similar spots were produced within 24 hours when moistened pieces of the liner were laid on other pears of the same lot. No discoloration was found under cotton moistened with dis-

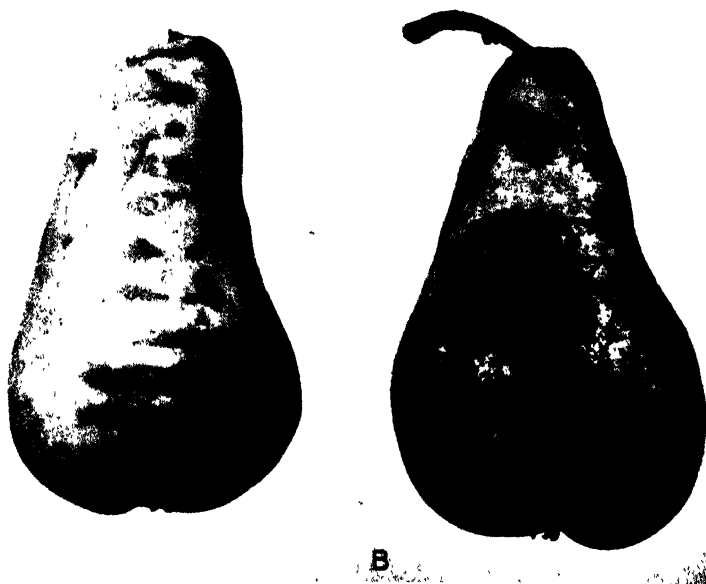


FIGURE 1. -A, Discoloration on a Bosc pear that was in contact with a corrugated paper liner in a commercial shipment. B, Injury produced on a Bosc pear by a 0.1 N solution of sodium hydroxide. This injury was similar to that produced by corrugated paper liners and alkaline solutions of sodium silicate.

tilled water. It was observed at this time that in all cases the browning was most pronounced on russeted areas.

LATER EXPERIMENTS

After these preliminary investigations no further work was done on the problem until the winters of 1929-30 and 1930-31, when an extensive series of tests was made with six varieties of pears. Seventeen samples of corrugated paper from several sources, 1 sample of excelsior-filled pad, and 5 grades of sodium silicate were used in the tests.

VARIETIES OF PEARS USED

The varieties of pears used in the investigations were P. Barry, Winter Nelis, Bosc, Clairgeau, Bartlett, and Anjou, all of which were grown in Oregon or California. A considerable portion of the skin of P. Barry, Winter Nelis, and Bosc is covered with russet, some speci-

mens being entirely russeted. Clairgeau is a smooth-skinned variety, but the lenticels are conspicuously russeted. Bartlett and Anjou are smooth-skinned, although some specimens of the latter have a russeted area around the blossom end. The region in which the fruit was grown made no significant difference in the results obtained.

No attempt was made to duplicate all conditions with all varieties. Winter Nelis received all treatments. Fruit of the Bosc and Anjou varieties was subjected to most of the test conditions. Bartlett and Clairgeau were used with a few of the paper liners and silicate solutions; P. Barry was used with the paper liners only.

SODIUM SILICATE SOLUTIONS

The five brands of sodium silicate (A, B, C, D, and E) were made up in solutions of five different strengths, namely, 0.1, 0.5, 1, 5, and 10 percent. To test the effect of these, a piece of absorbent cotton about 1 inch square was moistened with the solution and placed on the fruit. The fruit was then placed in moist chambers and most of it was held for 15 days at 40° F. A few lots were held at 32° and 70° to 80° for various lengths of time.

Table 1 shows typical results obtained with solutions of sodium silicate on Winter Nelis, Bosc, and Anjou pears held at 40° F. for 15 days after treatment. The injury consisted of a brown spotting similar to that observed on affected fruit on the market.

TABLE 1.—*Injury produced by various solutions and brands of sodium silicate on pears of three varieties held at 40° F. for 15 days*

Variety	Brand of sodium silicate	Injuries produced by indicated concentrations of sodium silicate solution					
		0.5 percent		1 percent		5 percent	
		Pears	Injury	Pears	Injury	Pears	Injury
		Number		Number		Number	
Winter Nelis.	A	4	None	2	Both moderate	4	All severe
	B	4	3 severe, 1 moderate	4	2 severe, 2 moderate	4	Do
	C	4	3 moderate, 1 slight.	4	All severe.	4	Do
	D	3	2 moderate, 1 none.	4	2 moderate, 2 slight	4	Do.
	E	4	1 moderate, 3 slight.	3	All moderate.	4	Slight.
Bosc	B	3	1 slight, 2 none.	4	3 severe, 1 moderate.	4	All severe
	C	3	do	4	None	4	Do
	E	4	None	4	2 slight, 2 none.	4	Do
	B	4	do	4	None	4	2 none, 2 severe on russeted portion only
Anjou	C	4	do	4	do	4	1 none, 3 trace on russeted portion only.
	E	4	do	4	3 none, 1 trace on russeted portion.	4	None.

Solutions of the E brand silicate only were used on the Clairgeau variety. The only injury was a slight discoloration on a russeted fruit, caused by the 5 percent solution.

None of the solutions used had any effect on Bartlett pears at room temperature, about 70° F. No tests were run at other temperatures.

All the concentrations of sodium silicate solutions of each of the five brands were used with Anjou, Winter Nelis, and Bosc pears at room temperature and with Winter Nelis and Anjou at 32° F. No injury was produced on Anjou, and the results with Winter Nelis and Bosc were similar to those shown for these varieties in table 1.

A few drops of each of the five brands of undiluted sodium silicate were poured out on watch glasses and the fruit was placed directly in this liquid. The result was a brown to almost black injury of the skin and flesh, which was severe on Winter Nelis pears and somewhat

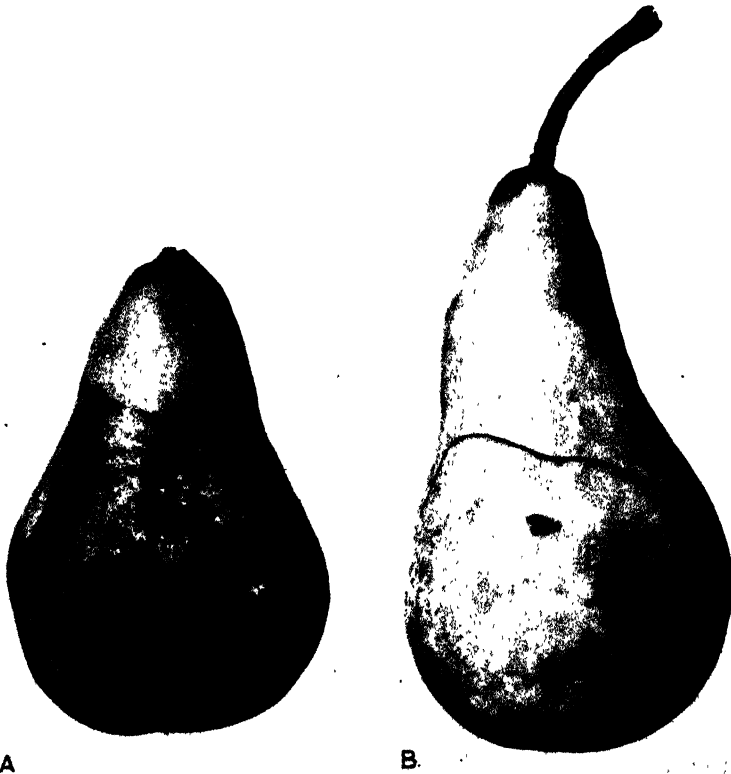


FIGURE 2.—Bosc pears treated with 5 percent sodium silicate. A, Silicate solution not neutralized, B, silicate solution neutralized with sulphuric acid. Areas that were in contact with test solutions are outlined by dark lines.

less so on Anjou. In some cases the discoloration extended into the flesh for a quarter of an inch.

The alkalinity of the various sodium silicate solutions was determined by titrating a 1 percent solution with sulphuric acid, methyl orange being used as an indicator. The results are presented in table 2.

The results of the tests just described furnished some evidence that the discoloration was caused by free alkali in the solution. To obtain further information on this point, enough sulphuric acid was added to the various solutions of sodium silicate used to just neutralize them to methyl orange. The neutralized sodium silicate solutions were

used in the same concentrations, on the same varieties, and under the same conditions as the alkaline sodium silicate solutions previously described. Although the unneutralized solutions caused some injury in concentrations as low as 0.5 percent, and in most instances severe injury to certain varieties, in concentrations of 5 percent the neutralized solutions produced no injury on any of the fruits (fig. 2).

TABLE 2.—*Approximate percentage of alkalinity of sodium silicate solutions*

[Alkali calculated as NaOH, using figures obtained by titrating silicate solutions with 0.1 N H₂SO₄, indicator, methyl orange]

Brand of sodium silicate	0.1 percent solution	0.5 percent solution	1 percent solution	5 percent solution	10 percent solution	Undiluted (calculated)
A.	0.02	0.10	0.21	1.05	2.09	20.9
B.	.037	.18	.37	1.83	3.67	36.7
C.	.015	.08	.15	.76	1.53	15.3
D.	.016	.08	.16	.81	1.62	16.2
E.	.027	.14	.27	1.37	2.74	27.4

SODIUM HYDROXIDE SOLUTIONS

In order to obtain additional information on the relation of alkalinity to the injury, solutions of sodium hydroxide were applied to pears by means of absorbent cotton in a manner similar to that described for sodium silicate solutions. The results obtained at 40° F. are shown in table 3. The injury produced could not be distinguished from that produced by the sodium silicate solutions, paper liners, or the seams of excelsior-filled pads (fig. 1, B). Dilute acid solutions produced typical acid injury, which was easily distinguished from the injury caused by alkali.

TABLE 3.—*Injury produced by solutions of sodium hydroxide of different normalities on pears of four varieties held at 40° F. for 15 days*

Normality of solution	Winter Nelis		Bosc		Anjou		Chaugeau	
	Pears	Injury	Pears	Injury	Pears	Injury	Pears	Injury
0.3 N (1.2 percent)	Number 4	Very severe	Number 4	2 severe, 2 moderate	Number 4		Number 3	Slight on russet portion only
.2 N (0.8 percent)	4	Severe	3	Moderate	4	Slight on russet portion only		
.1 N (0.4 percent)	4	do.	4	2 severe, 2 moderate	4	None	3	2 none, 1 trace on russet portion only
.03 N (0.12 percent)	4	2 severe, 2 moderate	2	1 severe, 1 trace	4	do.	3	None
.01 N (0.04 percent)	4	do.	4	1 trace, 3 none				

EXCELSIOR-FILLED PADS

Excelsior pads covered with paper are used to some extent in pear boxes as a protection against bruising, especially with tender varieties. The edges of the paper are lapped and fastened together with an

adhesive, usually sodium silicate, and the seams thus made may cover a quarter of the area on one side of the pad.

Moistened pieces of these seams produced injury on Bosc, Winter Nelis, and P. Barry pears (fig. 3, B). This injury was similar to, but usually more severe than, that produced by paper liners. The alkalinity of the seam, determined as previously described for the paper liners, was 2.35 percent, calculated as NaOH. A water extract of the excelsior caused no discoloration of the pear skin.

CORRUGATED-PAPER LINERS

Pieces of corrugated-paper liners about 1½ inches square were moistened with distilled water and placed in moist chambers and pears were laid on them. Only enough water was used to make the

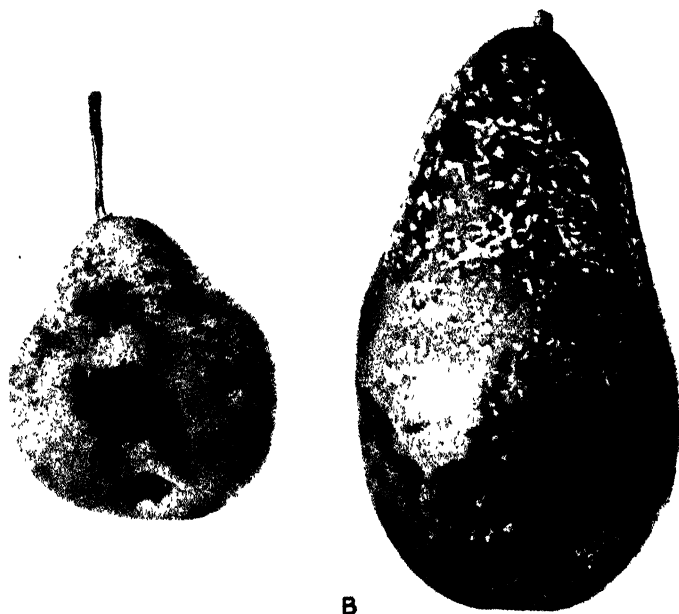


FIGURE 3 —A, Discoloration on a Winter Nelis pear, produced by placing it in contact with a moist section of corrugated-paper liner; B, P. Barry pear taken from a commercial shipment of pears in Chicago, showing discoloration on the portion that lay in contact with an excelsior-filled pad the seams of which contained sodium silicate

paper moist, in order to avoid the possibility of washing out any soluble substances that the paper might contain.

The alkalinity of the various corrugated papers was determined as follows: A piece of each paper, containing about 12 to 15 square inches, was weighed and then placed in distilled water for 24 hours. The resulting solution was then titrated with 0.1 normal sulphuric acid, methyl orange being used as an indicator. The alkalinity by weight was calculated as sodium hydroxide.

The amount of discoloration caused by different samples of corrugated-paper liners at 40° F. is given in table 4. The duration of the treatment was 15 days.

TABLE 4.—Discoloration produced on pears of 4 varieties by contact with corrugated-paper liners for 15 days at 40° F.

Paper liner no.	Adhered with—	Alkalinity expressed as NaOH		Winter Nels		Anjou		Bosc		Clargeau	
		Percent	Number	Pears	Injury	Pears	Injury	Pears	Injury	Pears	Injury
1	Sodium silicate.	0.91	4	4	1 none, 3 moderate.	Number	4	3	1 severe, 2 moderate.	Number	3
2	Flour dextrin paste.	.08	4	4	None.	4	do.	3	None.	3	None.
3	do.	.09	4	4	do.	4	do.	3	None.	3	None.
4	Sodium silicate.	1.06	4	4	Trace.	4	do.	3	Moderate.	3	do.
5	do.	.83	4	4	2 severe, 2 moderate.	4	3 none, 1 moderate on russet portion only.	3	do.	3	do.
6	do.	1.22	3	3	2 severe, 1 trace.	4	None.	3	Slight.	3	Slight.
7	do.	.53	3	3	do.	4	None.	3	None.	3	None.
8	do.	.82	3	3	1 none, 1 moderate, 1 severe.	4	None.	3	do.	3	do.
9	do.	.49	4	4	2 none, 1 trace, 1 moderate.	4	None.	3	do.	3	do.
10	do.	1.43	4	4	1 trace, 3 moderate.	4	None.	3	None.	3	None.
11	do.	.79	4	4	3 none, 1 trace.	4	do.	3	do.	3	do.
12	do.	1.60	4	4	1 moderate, 3 severe.	4	do.	3	Moderate.	3	Moderate.
13	do.	.76	4	4	None.	4	do.	3	None.	3	None.
14	do.	.05	4	4	do.	4	do.	3	do.	3	do.
15	Flour dextrin paste.	.73	4	4	3 trace, 1 moderate.	4	3 none, 1 trace on russet portion only.	3	Trace.	3	Trace.
16	Sodium silicate.	.76	4	4	3 moderate, 1 severe.	4	do.	3	do.	3	do.
17	do.	.63	4	4	Severe.	4	None.	3	do.	3	do.
Check	Moist filter paper.		4	4	None.	4	do.	4	None.	4	None.

3 2 none, 1 slight on russet portion only.

Results similar to those given in table 4 were obtained with Anjou, Winter Nelis (fig. 3, A), and Bosc pears at room temperature (about 70° F.), and with Winter Nelis and Anjou at 32°. Discoloration was produced on P. Barry with paper liner no. 6 at room temperature. None of the papers used produced injury on Bartlett pears.

MOISTURE

To test the effect of moisture in the package during the storage period, Winter Nelis pears were packed in two standard pear boxes with a paper liner that had been found to cause discoloration. The liner in one box was moistened; that in the other box was left dry. About one half of the pears in each box were wrapped in ordinary fruit wrappers. Both boxes were then left at 32° F. for 42 days, after which they were examined. The results are given in table 5.

TABLE 5.—*Effect of moisture in the discoloring of Winter Nelis pears by paper liners, during a storage period of 42 days at 32° F.*

Packing conditions	Pears	Effect on pears at area in contact with paper liners
	<i>Number</i>	
Liner moist		
Pears wrapped	14	All badly discolored
Pears unwrapped	13	All badly discolored, slightly more than in wrapped fruit above
Liner dry:		
Pears wrapped	14	None
Pears unwrapped	14	1 pear discolored

REMOVAL OF SPOTTING WITH HYDROCHLORIC ACID

The spotting produced by paper liners was almost completely removed from Winter Nelis pears by allowing the fruit to remain in a 1 percent solution of hydrochloric acid for 1 hour. No apparent removal of the spotting was noticed after 24 hours when pears were placed in water alone.

DISCUSSION OF RESULTS

The typical brown discoloration that has been observed on the market and described earlier in this paper was produced experimentally on russeted varieties of pears by seams of excelsior-filled pads and by corrugated-paper liners when sodium silicate was used as an adhesive. This injury was also produced on russeted pears by solutions of sodium hydroxide and commercial sodium silicate. The injury was not produced by corrugated-paper liners in which flour-dextrin paste had been used as the adhesive instead of sodium silicate. Each of the corrugated-paper liners in which sodium silicate had been used as an adhesive produced the discoloration at least once on either Winter Nelis or Bosc pears during the series of tests.

EFFECT OF RUSSETING

The injury produced seems to be associated with the russetting on the fruit. The sodium silicate solutions, corrugated-paper liners, excelsior-filled pads, and sodium hydroxide solutions produced injury in these experiments on the russeted varieties, namely, Bosc,

Winter Nelis, and P. Barry. The injury on these varieties was most severe on the more heavily russeted fruit. Practically no injury was produced on the smooth-skinned varieties, although a small amount of discoloration was sometimes produced on the russeted areas of Clairgeau and Anjou pears. As heretofore mentioned, the discoloration has never been noticed on the market on smooth-skinned varieties.

Russetting results from a cracking and weathering of the epidermis and an increased development of the corky parenchyma beneath.² Fruit russeted as a result of frost injury wilts rather rapidly,³ which indicates that the skin of a russeted fruit is more permeable to water or water vapor than that of a smooth-skinned fruit.

The brown discoloration on russeted pears probably is caused by the action of the solution on the corky parenchyma or periderm which has been exposed by the disappearance of the epidermis, although it may result from the penetration of the solution into a few layers of cells of the flesh.

EFFECT OF ALKALINITY

The discoloration noted on pears in the various experimental lots of fruit and on those observed on the market had very much the appearance of plant tissues browned by ammonia or other alkalis. It seemed possible, therefore, that the browning produced when certain varieties of pears come in contact with corrugated paper put together with sodium silicate might be caused by free alkali in the silicate. The results of the experimental work described herein make it apparent that the brown discoloration is actually caused in this way. The following facts form the basis for this conclusion:

Although solutions of sodium silicate, in some cases in concentrations as low as 0.5 percent, produced injury, no injury was produced in any case when these solutions were neutralized, even when a 10 percent solution of the silicate was used.

Typical brown discoloration was produced by solutions of sodium hydroxide in concentrations as low as 0.01 N (0.04 percent).

Only the samples of corrugated-paper liners and the seams of excelsior-filled pads that contained sodium silicate produced the discoloration. These were of high alkalinity as compared with the corrugated pads in which some other adhesive was used and by which no discoloration was produced.

Although not conclusive in itself, the removal of the discoloration by a 1 percent solution of hydrochloric acid gives further evidence that the discoloration is due to alkalinity.

EFFECT OF MOISTURE

The humidity within a box of pears is probably high enough at times to cause some of the sodium silicate contained in corrugated-paper liners or the seams of excelsior-filled pads to go into solution and thus be carried to the skin of the fruit, where it produces the discoloration. The moisture probably results largely from condensation produced by the changes in temperature to which boxes of pears are normally subjected. Probably some moisture is also given off by the fruit itself.

HYDROLYSIS OF SODIUM SILICATE

It is realized that when enough acid is added to a sodium silicate solution to render it approximately neutral, the condition established

² GARDNER, V. R., BRADFORD, F. C., and HOOKER, H. D. THE FUNDAMENTALS OF FRUIT PRODUCTION. 686 p., illus. New York. 1922.

³ PADDOCK, W., and WHIPPLE, O. B. FRUIT GROWING IN ARID REGIONS: AN ACCOUNT OF APPROVED FRUIT-GROWING PRACTICES IN THE INTER-MOUNTAIN COUNTRY OF THE WESTERN UNITED STATES. 395 p., illus. New York. 1914.

is probably not permanent because of the hydrolysis of the silicate and the setting free of more alkali in the solution.⁴ The extent of this hydrolysis was evidently small, however, during the course of these tests. The fact that solutions of sodium silicate or sodium hydroxide produced the discoloration, whereas "neutralized" solutions of sodium silicate did not, is strong evidence that for the duration of the experiments described there was little or no hydrolysis and consequently little or no release of free alkali into the sodium silicate solution after "neutralization."

SUMMARY

A brown discoloration of russeted varieties of pears is described.

The injury was produced experimentally by placing fruits in contact with corrugated-paper liners and the seams of excelsior-filled pads containing sodium silicate, and by applying to the fruits weak solutions of commercial sodium silicate and of sodium hydroxide. Neutralized solutions of sodium silicate and paper liners put together with some adhesive other than sodium silicate did not produce the injury.

Alkaline substances contained in sodium silicates apparently are the direct cause of the injury as it occurs in commercial lots of fruit.

In many cases there is apparently sufficient moisture within boxes packed with pears to cause some of the alkaline substances contained in paper liners put together with sodium silicate to go into solution and produce the injury.

⁴BOGUE, R. H. THE HYDROLYSIS OF THE SILICATES OF SODIUM *Jour Amer Chem Soc* 42 2575-2582. 1920.

VITAMINS C AND A IN BLUEBERRIES ¹

By C. R. FELLERS, research professor, and P. D. ISHAM, research fellow, Department of Horticultural Manufactures, Massachusetts Agricultural Experiment Station ²

INTRODUCTION

Blueberries are very widely distributed in the United States, Canada, and Newfoundland. *Vaccinium corymbosum*, the high-bush, cultivated type, and *V. pennsylvanicum*, a low-bush, dwarf type, are the common species growing in northern New England, the Canadian Maritime Provinces, and Newfoundland. Several minor species are also of importance in some localities.

Blueberries ripen over a period of several months and keep remarkably well. Fresh blueberries are extensively used as food and are also canned and frozen, and after preservation are used commercially in the bakery, preserve, and jelly industries. Blueberries thus constitute an important item in the diet of large numbers of people over a wide territory.

As no reports on the vitamin content of blueberries had come to the writers' attention, animal feeding tests designed to evaluate this fruit for its vitamin C and A content were accordingly begun in the summer of 1931. The results of these tests are reported in this paper.

EXPERIMENTAL PROCEDURE

VITAMIN C IN BLUEBERRIES

The method of Sherman, La Mer, and Campbell ³ was used, with the exception that the amounts fed were proportioned to weight of the guinea pigs, i.e., a 400-gram animal was fed four thirds as much as one weighing 300 g. The animals were all young and healthy, weighed between 280 and 325 g, and were kept in individual cages. The basal ration consisted of 58 percent equal parts of rolled oats and wheat bran, 30 percent of vitamin C free baked skim-milk powder, 10 percent butterfat, 1 percent each of cod-liver oil and salt. This basal ration and water were kept before the animals at all times.

Three guinea pigs normally were used at each feeding level. At the end of the feeding period all animals were chloroformed and carefully examined for lesions of scurvy. Negative controls died in from 26 to 33 days with an average Sherman scurvy score of 16.

Native western Massachusetts high-bush blueberries, *Vaccinium corymbosum*, were used in both 1931 and 1932 in the tests on fresh blueberries. Since the latter were available for only 85 and 80 days, respectively, in 1931 and 1932, the feeding tests were limited to this period.

Frozen native, high-bush blueberries, *Vaccinium corymbosum*, were from the same lot that was fed fresh in 1932. These were wrapped in cellophane, packed in 1-pound waxed-paper cartons, and frozen at Gloucester.

¹ Received for publication Mar. 6, 1933; issued August 1933. Contribution no. 165 of the Massachusetts Agricultural Experiment Station.

² The writers are indebted to the Birdseye Laboratories, Gloucester, Mass., for freezing, storing, and shipping the frozen samples from Massachusetts that were used in 1932.

³ SHERMAN, H. C., LA MER, V. K., and CAMPBELL, H. L. THE QUANTITATIVE DETERMINATION OF THE ANTISCORBUTIC VITAMIN (VITAMIN C). Jour. Amer. Chem. Soc. 44: 165-172, illus. 1922.

ter in the Birdseye multiplate freezer at about -30° F. and stored at 0° for 5 to 8 months. Samples for vitamin assay were shipped weekly with solid carbon dioxide and were still solidly frozen upon arrival at the laboratory. The cartons were held at -0° until just before using.

The Maine blueberries were the low-bush species, probably *Vaccinium pennsylvanicum* and were quick frozen by the Birdseye method at Winslows Mills, Me., during August in 1931 and 1932. No sugar was added. The berries were wrapped in cellophane and packed in 1-pound paraffined-paper cartons. Supplies packed with solid carbon dioxide were sent to the laboratory weekly. The Newfoundland blueberries were also the low-bush type, probably *V. pennsylvanicum*, and were quick frozen by the Birdseye method at St. Johns. They were packaged, stored, and shipped similarly to the frozen blueberries from Maine.

Blueberries were canned in both 1931 and 1932. In 1931 both the native high-bush and the low-bush types were canned. The high-bush native blueberries were blanched for about 30 seconds in boiling water, filled into spring-clamp glass jars, processed in a water bath at 212° F. for 8 minutes, and sealed. The low-bush variety was treated in the same way, except that the jars were vacuum-sealed at a partial vacuum of 25 inches of mercury. These berries were grown on Cape Cod, whereas the high-bush berries were grown in Amherst. In 1932 the high-bush variety from Amherst was again canned. This time the fruit was not blanched but was filled directly into glass jars, covered with a previously boiled hot 10 percent sugar sirup, vacuum-sealed at a partial vacuum of 20 to 22 inches of mercury, and processed at 212° for 8 minutes. The cooled jars had partial vacuums of 15 to 18 inches of mercury.

In every case the guinea pigs ate the fresh, frozen, or canned blueberries. A few animals that disliked the fruit were discarded early in the experiment and new animals substituted for them.

VITAMIN A IN BLUEBERRIES

A preliminary test of the vitamin A content of Maine quick-frozen blueberries was run according to the Sherman and Burtis technic.⁴ Depleted rats were fed the vitamin A free diet supplemented by 1 and 3 g of blueberries, but no significant evidence of protection was secured at either level. Although too scanty data were obtained to render these results quantitative, the agreement is such that the conclusion that blueberries contain but a trace of vitamin A, less than 0.3 unit per gram, seems justified.

RESULTS

The results of the experiments on the vitamin C content of blueberries are reported in table 1.

Data on two successive crops of *Vaccinium corymbosum*, high-bush blueberries, show a minimum protective dose of 4 to 5 g. There is little or no loss due to quick freezing. The canning method may be of importance in the retention of vitamin C. For example, the unblanched vacuum-canned blueberries were far superior to the same variety which were canned a year earlier but which were blanched for 30 seconds in boiling water and sealed without vacuumization.

⁴ SHERMAN, H. C and BURTIS, M. P. FACTORS AFFECTING THE ACCURACY OF THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Biol. Chem. 78: 671-680. 1928.

TABLE 1.—Vitamin C in blueberries

Source and treatment of blueberries	Year	Animals	Quantity fed	Change in weight	Average survival period	Scurvy score	Remarks
		Number	Grams	Grams	Days		
	1931	3	3	24	85	7	Moderate scurvy, experiment terminated in 85 days.
Native, high-bush, fresh.	1931	3	5	110	85	0	Do.
	1932	2	3	40	80	3	Moderate scurvy; experiment terminated in 80 days.
Native, high-bush, frozen ----	1932	2	5	349	80	0	Do.
	1932	3	7	346	90	0	
	1932	3	11	322	90	0	
Maine, low-bush, frozen ---	1931	3	4	-120	33	11	Severe scurvy.
	1931	3	8	-109	65	8	
	1932	3	10	149	90	3	Slight scurvy.
Newfoundland, low-bush, frozen	1932	3	14	156	90	1	Trace scurvy.
	1931	3	4	-143	37	12	Severe scurvy.
Native, high-bush, blanched, canned	1931	3	8	-115	43	11	Do.
	1931	3	4	-117	43	11	Do.
Native, low-bush, blanched, vacuum canned	1931	3	6	-115	60	16	Do.
	1931	3	4	-65	39	16	Do.
Native, high-bush, not blanched, vacuum canned	1931	3	8	-114	38	14	Do.
	1932	3	5	360	90	0	No scurvy.
Negative control...	1932	3	10	311	90	0	Do.
	1932	3	0	-130	30	16	Severe scurvy.

Too few controlled canning tests were made to justify conclusions relative to the effect of canning methods, yet it is significant that one of the methods used did conserve the vitamin C.

Although the low-bush blueberries, *Vaccinium pennsylvanicum*, were not assayed in the fresh state, nevertheless, inasmuch as quick freezing followed by storage at 0° F. did not injure the vitamin C content of cranberries,⁵ strawberries,⁶ or the high-bush blueberries, the same process probably caused no loss in the low-bush species.

The results obtained in using the low-bush species indicate considerable variation, both seasonal and regional. The 1932 Maine crop apparently contained more vitamin C than did that of 1931, but even this species is but 25 to 35 percent as active as the high-bush species, the minimum protective portion being 15 to 16 g. There is apparent also a slight difference between the vitamin C potency of the low-bush blueberries grown in Maine and those grown in Newfoundland. The results indicate that the Newfoundland berries are but 75 to 85 percent as potent as the Maine berries.

Only one sample of low-bush blueberries was canned. At the 8-g level, the highest amount fed, very little protection from scurvy was obtained.

SUMMARY

Vaccinium corymbosum, the high-bush blueberry, is a good source of vitamin C. Quick freezing and certain methods of canning did not significantly injure the vitamin C content.

Vaccinium pennsylvanicum, the low-bush blueberry contains only 25 to 35 percent as much vitamin C as does *V. corymbosum*. Only a trace of vitamin A is present in *V. pennsylvanicum*.

⁵ FELLERS, C. R. NUTRITIVE VALUE OF CRANBERRIES. Amer. Jour. Pub. Health 23: 13-18. 1933.
⁶ — and MACK, M. J. Indus. and Engin. Chem. 25: ———— 1933. (In press.)

THE LAW OF DIMINISHING RETURNS IN AGRICULTURE¹

By P. E. McNALL

Professor, Department of Agricultural Economics, Wisconsin Agricultural Experiment Station

INTRODUCTION

A correct understanding of the law of diminishing returns both as to its application, as well as to its statement as a principle, is fundamental in any study designed to measure the effect of increasing units of an input factor upon the output whether this output be in the form of plant growth or animal production.

Immediately following Von Liebig's emphasis of the need by plants of various mineral nutrients necessary for growth, attempts were made to bring under some law the relation existing between added units of any input factor necessary for plant growth and the additional outputs caused by those additional units. Von Liebig developed his law of the minimum, namely, that the productivity of a field is in direct relation to the necessary constituent contained in the soil in the smallest quantity (8, p. 105)². In this law he recognized not only the importance of the limiting factor in plant growth but also the necessity of a balanced plant-food supply. The law itself, however, was not correctly stated, and it took the thought and research of four generations of soil chemists to develop a sufficient body of data under carefully worked out control conditions to show (1) that the law as Von Liebig stated it was incorrect and (2) to make a correct statement of the law possible.

DISCUSSION

It was Mitscherlich (10, 11, 12, 13, 14, 15, 16, 17) preceded by Mayer (9) and Wagner (20) who, following years of experimentation, finally restated the law to express the diminishing increments³ of output with the addition of like units of any input factor. He first experimented with different forms of phosphoric acid fertilizer in pot experiments and showed that as the applications of either of the forms of phosphoric acid were increased the yield also increased, but at a decreasing rate. As the application of monobasic phosphate of lime was increased from 1 to 10 units, for example, the yield increased at the following rate (19):

Units applied	Increase in yield per added unit of fertilizer
1.0	44.3
2.5	10.8
5.0	7.2
10.0	2.0

¹ Received for publication Oct. 17, 1932, issued August, 1933.

² Reference is made by number (italic) to Literature Cited, p. 177.

³ "Increments" is used to indicate the added output, and "returns", total output.

Later experiments were performed with other fertilizing elements, and these were followed in turn by experiments in which the water and sunlight were varied. The experiment varying the amount of sunlight is interesting. Plants were grown in greenhouse pots shaded by nets varying from 2 to 10 in number for different pots, and the observed growth was compared with full growth in the open. Some of the results are shown in table 1. The increments of growth (or yield) in this instance showed, in general, a decreasing rate in each of the different plants used in the experiment.

TABLE 1.—*Growth increments of plants in greenhouse pots as related to increased intensity of sunlight*

Increase in intensity of sunlight ^a	Increment of growth or yield per unit of added light intensity			
	Oats	Peas	Buck-wheat	Lupins
0.025	3.2	1.9	1.6	0.6
.033	3.2	1.4	2	1.2
.044	3.9	1.6	2.2	.9
.063	2.1	8	1.4	8
.098	1.7	7	1.2	.6

^a In terms of sunlight in the open expressed as 1.00, 0.192 is the least intensity used in the experiment

It is needless to add to the number of experiments that may be referred to in the United States and in England as well as in Germany to prove the existence of this particular relationship, and it was this type of work which was positively accepted by scientists as proof of the inaccuracy of Von Liebig's statement of the law. It also showed that as any element necessary for plant growth became less (assuming the quantity in the soil to be less than the optimum) the yield was reduced at an increasing rate. Out of this came Mitscherlich's well-known law of the soil.

The next conspicuous development in the theoretical aspects of the law of diminishing increments was to give mathematical expression to the law. Experiments have demonstrated that the cause-result relationships are so certain that they can be shown with mathematical exactitude.⁴

Both Mitscherlich and Spillman, working independently and unknown to each other, showed, through empirical means, that input-output relationships for different plant and animal production assumed a certain form which is expressed by a logarithmic curve. This curve is so constructed that each point on the vertical axis bears the same relation to the maximum vertical rise when expressed in terms of the corresponding point on the horizontal axis as any other point on the axis. If, for example, a unit of any input factor, horizontal axis, results in a yield, vertical axis, of one half the possible maximum which this variant can cause, then the second unit of input will result in the production of one half the remainder; and the addition of a third unit of the input factor will yield one half the remaining possible production which this factor can cause (1, 19).

⁴ It should be remembered, however, that results can be predicted with any degree of accuracy only when all the variable factors affecting production or growth are known, and only those are varied whose effects are to be determined.

It is obvious that this curve represents diminishing output per unit of additional input and that exact expression can be given it. In each of these experiments, especially those performed by Mitscherlich, productions were obtained with the variation of a single element of plant growth. Nitrogen, or phosphorus, or sunlight was varied separately, but not two were varied simultaneously. It was in connection with the variation of more than one input factor that Mitscherlich indicated his inability to show and measure results. Baule, however, using some of Mitscherlich's data, deductively arrived at another mathematical expression which when plotted shows a sigmoid or S curve rather than the characteristic logarithmic curve so commonly accepted as the relationship existing between added units of input of any one factor and the corresponding additional outputs.

In working on this problem Baule expressed as 100 the maximum possible yield due to the variation of any single factor (1). He then determined the amount of the variable element necessary to produce 50 percent of the maximum, called the Baule unit. If x units of the variable produced 50 percent of the possible maximum, $2x$ units would produce 50 percent plus one half of 50 percent (remaining production) or 75 percent of the maximum, and $3x$ units would produce 75 percent plus one half of 25 percent or 87½ percent, etc. The tabular expressions of the Baule relationships of individual elements of plant growth are shown in table 2.

TABLE 2. - Baule relationships between individual fertilizing elements favoring plant growth

Input		Output in terms of maximum output	
Additional	Total	Additional	Total
1	1	$\frac{1}{2}$	$\frac{1}{2}$
1	2	$\frac{1}{4}$	$\frac{3}{4}$
1	3	$\frac{1}{8}$	$\frac{7}{8}$
1	4	$\frac{1}{16}$	$\frac{15}{16}$
1	5	$\frac{1}{32}$	$\frac{31}{32}$

It was shown that the same relationship would hold for any of the fertilizing elements, so that if the application of nitrogen were varied in one experiment while the application of phosphorus were varied in another it would be possible to make the units of the different kinds of fertilizers of such amounts that the first application in each case would result in an increase in growth equal to 50 percent of the possible maximum growth of the plant; and subsequent applications of the same sized unit would result as indicated in table 2. In other words the curve would be logarithmic.

But what would be the effect of equal doses if a combined unit of nitrogen and phosphorus were applied? It is obvious that the first dose of the combined units of nitrogen and phosphorus would not result in 50 percent plus 50 percent, or 100 percent of the total possible yield. It would be larger than if either had acted alone, but how much larger can be determined only by experimentation. The maximum result of the combined units would again be 100 percent, but this

100 percent seems to bear no known relationship to the "100 percents" representing the maximum possibilities of the respective plant-food elements composing this unit.

If, for example, the greatest possible increase in growth caused by the application of nitrogen to a soil is 40 bushels and the greatest possible increase in yield caused by the application of phosphorus is 30 bushels for the particular soil and climatic conditions in the experiment, it does not necessarily follow that the 100 percent figure for the effect of the combined elements would be 40 plus 30 or 70 bushels. Neither is it logical to expect as small an amount as is received by the application of either of the separate elements alone. Obviously the 100 percent point for the maximum effect of the combined factors would be somewhere between these two points.

Wherever this point is located Baule speaks of it as 100 percent and in order to obtain the relative effect of the simultaneous application of two fertilizing elements he multiplies the proportional yield obtained by one by that of the other. If the first application of nitrogen gives one half of the possible maximum for that element and the first application of phosphorus gives one half of its greatest maximum the effect of the simultaneous application of these two units would be one half times one half, or one fourth of the greatest possible growth, of the combined effects. The second dose of the combined elements would result in three fourths of three fourths or nine sixteenths of the greatest possible growth, and the third dose would result in seven eighths of seven eighths or forty-nine sixty-fourths of the greatest possible growth, of the combined factors. The result of this combination would not be a logarithmic curve but would be a sigmoid or S curve.

The practical significance of this development would be that, whereas the first application (or first unit available for plant growth) of any single fertilizing element or factor of production causes a greater relative growth than any subsequent application of a like unit, the first application of a unit composed of two growth factors would have relatively less effect upon plant growth than would some of the following doses. It necessarily follows from this deductive formulation by Baule that the greater the number of factors of plant growth simultaneously applied, the less will be the relative effect of the first units applied and the greater will be the relative effect of some of the following units (13).

It was also brought out by Mitscherlich that the greater the number of variants simultaneously applied, the more delayed will be the point at which the greatest relative effect will take place and the later in the plant growth will the point of diminishing increments occur.

This general relationship is shown graphically in figure 1.

Mitscherlich accepts this as the true relationship between the input and output factors when more than one causal factor comprises the input dose. He shows that it applies to plant growth when the plant development is recorded from a seedling. In his experimentation with sorghum plants in pots under glass the effect of applying equal "quantities of factors of growth" (13) composed of several fertilizing elements was shown upon the "accretion" or growth of the plants. Equal growth periods were determined by observing the number of days required for a radish seed to germinate and grow to

the point of formation of its second pair of leaves. He hoped in this manner to equalize variations in growth conditions caused by differences in sunlight, humidity, temperature, etc., so that each growth period would have as nearly equal as possible the amounts of those factors of growth which he wished to hold constant. This resulted in a variation of from 11 to 26 days in the time required for different groups of radishes to develop the same amount of growth. Six of the pots were harvested at each of the successive growth periods until some of the plants had grown nearly 6 months. The amount of growth was obtained by determining the grams of dry substance in each "section of growth", or equal growth period.

When these weights are plotted using equal growth periods as the horizontal or variable axis it shows a typical S or sigmoid curve, which is interpreted by Mitscherlich as proving, or at least demonstrating,

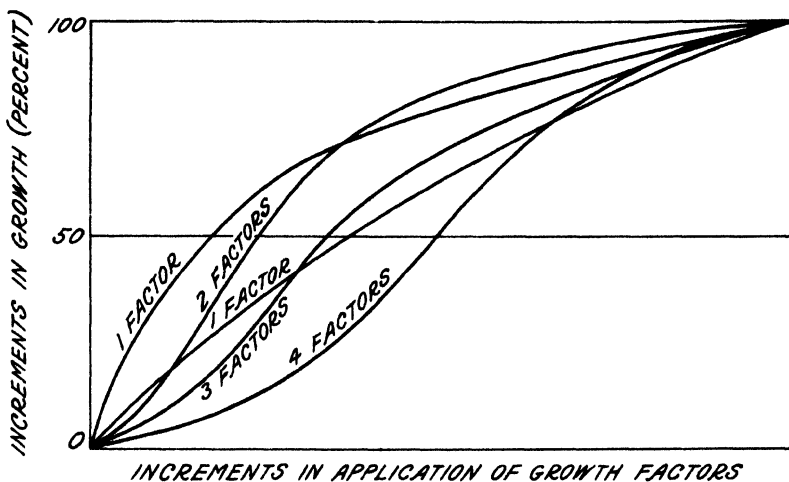


FIGURE 1.—General relationship between the application of various numbers of growth factors and the stage of plant growth at which the point of diminishing returns occurs.

the increasing and decreasing increments of plant growth per unit of input.

Several questions present themselves before this relationship can be accepted as a substitute for the law of diminishing increments or law of physiological relations. It is quite commonly recognized that plant (or even animal) growth is slow in its initial stages of development. Leaf surface for photosynthesis and root area for plant-food absorption are both limited so that only a small amount of plant growth can take place in a given period of time (or growth period). As the plant becomes larger and functional surfaces increase, growth normally becomes more rapid until the plant reaches approximate maturity. At some point in the latter part of the development of the plant the growth is again retarded. This relation of the growth of a plant to the time element has been called the "grand growth curve of plants" and is stated as follows: "Crop growth is accomplished in two principal stages, the first stage being characterized by an increasing rate of growth and the second by a decreasing rate * * *" (22, p. 47). Growth of this character will take place so long as there are

plant-food elements and other factors of growth in sufficient amounts to mature the plant. Growth will be greater in every stage of its development if larger quantities of growth factors are available, but the increasing and decreasing relationships will continue. This particular phenomenon, or characteristic, is not a function of depleted or changing growth factors but is a function of time alone.

Because of this characteristic of plant growth it will be necessary to call into question any results which presume to measure the effect upon crop production of additional applications of some growth factor, as fertilizers, when they are expressed in terms of growth periods. If the fertilizing elements of nitrogen and phosphorus are being applied in increasing amounts, it will be necessary to determine just how much growth is obtained through the addition of each dose of the combined unit. The effect of the first dose upon plant growth cannot be measured until the plant has taken up all of the dose that is biologically possible. The application of the second dose—or two doses to the second plant—must be treated in a manner similar to the first, and so on until maturity is reached. It is probable that when the results of an experiment of this type are plotted it will be found that the first application of nitrogen and phosphorus gives the greatest growth and that each additional dose results in slightly less growth than does the preceding dose.

Experimentation has shown that in some circumstances initial increments of some plant-food element, as nitrogen, when added to a soil deficient in that element may result in no increase in the availability of that element for plant growth—the added amounts being locked in the soil in the form of salts not readily available for plant growth. This results in small increments in plant growth for the initial applications of the fertilizing element. Although experimentation with the simultaneous application of two fertilizing elements has not been recorded where the results are as indicated above, it may be expected that in certain soil and plant nutrient conditions the same relationship may be observed. No generalization should be made from a few instances of this nature, however, as they are more than offset by experiments showing the greatest increments with initial applications. Recognition must also be given to the difference between application and availability.

It should also be remembered that in each of these generalizations it is presumed that what holds for plant growth also holds for crop production. If diminishing increments are characteristic of plant growth under controlled conditions, it is presumed that diminishing increments will characterize crop production of the same plant when grown in larger areas.

It may be desirable to know whether a small dose of fertilizer will have relatively greater effect upon plant growth when applied at the time of seeding, when the plant is 2 inches high, or when the crop is nearly matured, but this is a different question from that of determining the effect of increasing units of fertilizer upon crop growth or production. It is necessary to know how crop yields will be affected with different growth conditions in order that the generalizations may be effectively applied to the industry of farming; and experiments based upon different stages of plant growth which admittedly are characterized by increasing and decreasing increments must be

properly interpreted with specific reference to this one characteristic if experiments are to be useful in the general production of the crop.

It should be remembered that the method of combining two or more variables is not limited to one mathematical statement. The only premise from which Baule could have developed the sigmoid curve was by assuming that the initial additions of a combination of input factors result in less production than do subsequent additions; while if still more additions of the input factor are made, production will be at a lessened rate.

Had Baule assumed that the initial application of units ⁵ of any combination of input factors results in the largest relative increase in production, and that subsequent units result in increases but at a decreasing rate, he could have paralleled the logarithmic curve shown by the use of one variable. Feed as well as fertilizer experiments which are sufficiently complete to throw light on this subject show the decreasing output characteristic.

Many experiments have been set up showing the effect of the functioning of the law of diminishing increments, or the law of the soil, as Mitscherlich expresses it, and the results have been generally accepted. But a few questions should be raised relative to some of the conclusions reached.

It has been shown at different times, for example, that this law functions in the application of water to a crop. Widtsoe and Merrill (21) show that when varying amounts of irrigation water are applied to wheat the yield of grain per acre-inch of water decreased consistently from 2.02 bushels per inch for 5 acre-inches of water to 0.77 bushel per inch for 50 acre-inches. A crop like corn which has greater yield possibilities in its present stage of plant development showed the same decline in relation to increasing amounts of water used. The yield of this crop ranged from 6.07 bushels per acre-inch of water with the application of 5 acre-inches, to 1.43 bushels per acre-inch with 55 acre-inches of water. Other crops, without exception, showed the same general trend in yields. Sugar-beet yields were reduced from 0.90 ton per acre-inch of water when 5 acre-inches were applied to 0.41 ton when 50 acre-inches of water were applied.

The authors sum up the presentation of the results of their investigations as follows (21, p. 114-115):

There is a great uniformity among the results obtained when different quantities of irrigation water are applied to the representative crops studied in these experiments. In practically every case, increasing the quantity of water increased the total yield of the crop, but in a smaller ratio as the maximum irrigation was approached. The more water that was used, the smaller was the return per acre-inch of water. So large was this decrease of the acre-inch equivalent that a serious waste of water is to be inferred. As farmers become better acquainted with this truth the problem of the best quantity of water to use must ever confront them with more and more force. Shall the irrigation farmer, having money invested in both land and water, attempt to produce the largest yield per acre or the largest yield per acre inch? Is there some point at which acre-yield and acre-inch-yield meet to produce the largest profits?

In this way the problem is thrown open to solution, and the discussion of the law of diminishing increments as a physical principle becomes identified with the economic concept of diminishing returns. How much land should a farmer use with any given quantity of water and with certain price relationships?

⁵ "Application" is used here to indicate availability.

Willcox (22), in using this same series of data concludes that there is but one way to move in the utilization of land and that "is toward extensive farming." In proof of this contention he uses the yields of wheat obtained when a given amount of water is applied (30 acre-inches in this case) to 1, 2, 3, 4, and 6 acres of land, the total yields being 47, 91, 130, 166, and 226 bushels from the respective acreages. From this he formulates a law of "increasing increments of yield (variable space)", which is the eighth of his laws of plant growth. "When the amounts of the material factors of plant growth are held constant while the areal space is increased, the yield of the crop is increased at an increasing rate" (22, p. 46).⁶

The discussion of this law is not very extensive; but if Willcox means that the aggregate production is increased as more units of a variable factor are applied to a fixed factor, it is self-evident and needs no comment. It matters not whether the product is expressed as a series of totals, or in terms of some fixed factor, as the acre-inches of water in this instance, because whenever an increasing total is expressed in terms of a fixed factor the series must show increasing ratios. If, as the writer believes, he is stating the law of increasing increments it is to be questioned either whether he has correctly interpreted the data or whether the conclusion concerning the application of the law to agriculture is correct.

If the production previously mentioned is expressed as yields per acre of land used it will show 47, 45, 43, 42, and 39 bushels per acre as the land area is increased from 1 to 6 acres. This is a typical illustration of decreasing average returns. If, on the other hand, each increment in production is expressed in terms of the added acreage the following series results, as shown in table 3.

TABLE 3.—*Relation between added acreage and production increments on irrigated land, the amount of water used remaining constant*

Total		Increase in -	
Acres	Yield	Acres	Yield per added acre
	<i>Bushels</i>		<i>Bushels</i>
1	47	1	47
2	91	1	44
3	130	1	39
4	166	1	36
6	226	2	30

It is here seen that as the land area is increased—the amount of water used remaining constant—production increases but at a decreasing rate per additional unit of input, or acres.⁷ This is another illustration of decreasing returns, rather than increasing returns as stated by Willcox.

⁶ The graphic presentation used by Willcox to show increasing returns is in error in that he uses acre-inches of water as the horizontal axis (causal or variable factor) rather than the increase in land surface, in acres. Had he plotted the aggregate yields against acres as this descriptive material suggests, he would have observed the diminishing returns characteristic.

⁷ It is conceivable that increasing increments will be in evidence if the amount of irrigation water applied is increased from 1 acre-inch, where no crop production will take place, to several acre-inches, where some crop production is obtained. As production increases from zero to something, the rate of increase is infinite and increasing increments must of necessity be evident. It is not so easily conceivable, however, that, after enough water is applied to obtain some production, further additions of equal amounts of water will result in more than proportionate or even proportionate increases in yield.

In making an application of this law to agriculture Willcox (22, p. 46) states:

The law of diminishing increments of yield is characteristic of extensive farming where land is abundant and the other growth factors, as water, fertilizer, labor, etc., are relatively scarce.

Quite the contrary is true.

Where land is relatively free much more land (per unit of other factors) will be used, but even in this instance extra land will show diminishing rather than increasing increments or returns. It would seem that Willcox has confused total returns with returns per unit of the variable and that he does not recognize the implications of the principle of the conservation of the scarce factor.

Another possible interpretation which Willcox may have had in mind is that as the acre-inches of water become less the yield will become increasingly greater per acre-inch of water. The construction of his chart would indicate this possible interpretation as would also his use of the words "space" and "area". It should be remembered, however, that the aggregate amount of water is held constant according to his premise, and that the varying factor is the area over which the 30 acre-inches of water is applied. The variant is not acre-inches of water but acres of land. When 30 acre-inches of water are applied to 2 acres of land instead of 1 the amount of water applied per acre must be one half as much as when applied to the 1 acre, and when the same amount of water is applied to 4 acres it must of necessity follow that but $7\frac{1}{2}$ inches per acre can be used. If the study is for the purpose of determining the effect of different amounts of water on crop production, the land area should be held constant, as was done by Widdsoe and Merrill in their experimentation.

When an irrigation farmer rents land and water rights he, either thinkingly or not, has determined on a working proportion of land to water. Let us assume that, after farming a few years, he finds his yields of wheat, with the applications of the different amounts of water, to be the same as shown in table 3. He also finds that he is limited to a specific quantity of water during the year. Will he use a few acres of land and apply the water at the rate of, say, 30 acre-inches per acre or will he use six times as many acres and apply 5 acre-inches of water per acre?

This question cannot be answered without knowing the rental charge (or cost) per acre of the land, the cost per acre-foot of water, and the price of the crop. The higher the land rent in proportion to the water cost the fewer the acres of land that will be used. If the farmer had to pay \$40 an acre for the rent of land and \$2.50 per acre-foot for water, a unit consisting of 1 acre of land and 30 acre-inches of water would cost him \$46.25. If wheat sold at \$1 per bushel he would receive \$47, or a net of \$0.75 above rent and water cost for each unit consisting of 30 acre-inches of water and 1 acre of land. If, on the other hand, he applied the same amount of water to a unit consisting of 6 acres of land the cost of the unit in this case would be \$246.25 while the returns would be but \$226, or \$19.75 less than rent and water cost for each unit of land and water. This may be an absurd spread of constant and variable costs, but it helps to illustrate the fact that at times it may pay the irrigation farmer to intensify his operations. If the rental charges were but \$10 an acre

and other costs and prices were as given above, it would doubtlessly profit the farmer to practice the more extensive type of production. In each instance production is accomplished by decreasing increments per unit of the variable; yet because of the change in relative scarcity (costliness) of one factor the farmer is forced to change the intensity of his farming operations. In no instance is there any indication of increasing increments.

Incidentally if the law of increasing increments of yields as expressed by Willcox does not hold, then his ninth law or "concentration law of growth factors" may well be called into question. This law states that: "In a normal environment the effectiveness of a given quantity of an external factor of crop growth is *inversely proportional* to its concentration per unit of space."

If one unit of fertilizer produces X units of product then, according to the writer's interpretation of this law, 2 units of fertilizer applied to the same area would produce $\frac{X}{2}$ units of the product per unit of fertilizer, or a total of $2 \times \frac{X}{2}$, or X units of product, and the application of 3 units of fertilizer would result in $\frac{X}{3}$ units of product per unit of fertilizer or a total of $3 \times \frac{X}{3}$, or X units. In other words, the greater the application of fertilizer per unit of land the less would be its effect per unit of fertilizer on crop growth. This is obviously in error and cannot be what the author had in mind. If he meant to imply that the influence of each added unit of fertilizer is relatively less than the effect of the preceding unit when applied to the same square foot or acre, this would appear to be a correct statement. It is a restatement of his seventh law, of diminishing increments of yield, however, and should not be set up as a new law.

Another restatement of the law of diminishing increments is found in the eleventh law, action law of growth factors, wherein Willcox (22, p. 55) accepts the definite logarithmic formulation of the law as developed by Spillman and Mitscherlich. It is stated as follows:

When a variable positive growth factor is increased by successive increments the increase in the yield of the crop due to any increment will be proportional to the difference between the total yield produced by the preceding increments and the maximum possible yield * * *.

A third interpretation which may be more in line with the discussion of the author is that, as a given quantity of a growth factor is spread over larger areas, it becomes less concentrated, and the total yield becomes greater per unit of this growth factor which is held constant. This, of course, is true but it is not the statement of a new law. Rather it is a restatement of the truism that the greater the total yield the greater the average yield per unit of any fixed factor.

This principle has also been applied to problems of digestion and metabolism as they affect livestock production. In the different experiments which the writer has reviewed the principle of diminishing increments applies. Planes of feeding work with different classes of livestock, including poultry (2), beef (5, 6, 7), and dairy production (3, 4, 18), all show the general tendency of additional outputs to decrease with added units of any input factor. The authors

have not usually set up their data to show this relationship; but wherever experiments are made with this principle in mind, this study should result not only in pointing the way to greater accuracy in research technic but the material itself should also stand up better under the scrutiny of logical interrogation.

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SOME FACTORS AFFECTING THE ELECTROLYTES OF THE STARCH GRANULES ¹

By D. W. EDWARDS, *junior chemist*, and J. C. RIPPERTON, *chemist*, Department of Chemistry, Hawaii Agricultural Experiment Station

INTRODUCTION AND REVIEW OF LITERATURE

The early workers on the constitution of starch regarded it as of purely organic composition ² (8, *pt. 1*) and of empirical formula $(C_6H_{10}O_5)_n$. While it was recognized that starch could not be prepared free from certain inorganic elements, these were regarded as adsorbed impurities, which could not be washed out by the levigation process used in the preparation of the starch. The structure of the starch granule was extensively studied, and many divergent theories proposed to explain its structure and method of growth. It was generally held, however, that it consisted of two parts, one the insoluble outer envelope called alpha amylose or amylopectin, and the soluble inner part called beta amylose or amylose.

Various workers attempted to remove the mineral elements in the starch by washing it with weak acids. Demoussy (3), in 1906, "deminerallized" rice starch with weak hydrochloric acid and found that it had the properties of a weak acid, comparable to carbonic acid, and formed compounds with metallic hydroxides and carbonates which were dissociable in water.

Samec (11, *p. 19*), in 1913, proposed the theory that the acidity of starch is due to phosphoric acid, which is combined as an amylophosphoric acid ester and reacts like a dibasic acid. The remaining valences of the phosphoric acid were found to be more or less completely satisfied with cations of the alkalies or alkaline earths. Washing with dilute acid was found to remove the cations but not the phosphoric acid. Autoclaving, however, was effective in splitting off the acid, and a phosphorus-free amylopectin could thus be prepared. The viscous properties of the original material, which had disappeared, could be regained by rephosphorizing.

Of the other mineral constituents, silica is always present in starch in considerable quantities and is considered as an essential part of the starch grain. The question as to whether or not silica also exists as an amylosilicic acid ester has been raised but, according to Samec, has not yet been proven. Traces of manganese, iron, aluminum, and the chloride and sulphate anions are also present, varying according to the extent of purification of the sample. Noncarbohydrate organic groups have recently been shown to be essential constituents of starch from certain genera of plants. Taylor (16, *p. 63*) has found that starch from some of the cereal plants contains fatty acids which are combined with the carbohydrate complex, while Samec (13) believes that in wheat starch there is a unit of nitrogen combined with the amylophosphoric acid.

¹ Received for publication Feb. 9, 1933; issued August, 1933.

² Reference is made by number (*italic*) to Literature Cited, *p. 190*.

Thus, the original concept of starch as a pure carbohydrate has gradually changed. Even though the amounts of these noncarbohydrate materials are small, sometimes only a few hundredths of a percent of the starch, they have a vital effect on its properties. Nor is the starch granule homogeneous. The phosphoric acid, for example, exists very largely in the amylopectin rather than the amylose. Sherman and Baker (15) found the amylopectin of potato to contain 0.156 percent P_2O_5 and amylose only 0.023 percent.

Numerous investigators have studied the effect of these different noncarbohydrate constituents on the physical properties of the starch. Samec (12) finds that the viscosity of his amylopectin is determined by its phosphoric acid content. Different cations produce very marked differences in the appearance and viscosity of the ester. Hydrogen produces a nearly transparent solution. The alkalis produce translucent, highly viscous solutions, while the alkaline earths give rise to opaque and much less viscous solutions.

The ease with which one cation will replace another in the raw starch granule has suggested this as a possible cause of the differences in the viscosity of different samples of starch of the same origin. Tryller (17) found that the salts contained in the tap water used in the manufacture of the starch caused nearly all of the potassium to be replaced by calcium. One of the authors (9) was able greatly to change the viscosity of edible-canna and potato starches by shaking with hundredth equivalent salt solutions. The valence of the cation was the determining factor, and a remarkable similarity of properties occurred within any one valence. That replacement was progressive, and in no instance complete, was shown by the continuous change in properties with the number of treatments and variations in concentration of the salt solutions.

The degree of base saturation of the amylophosphoric acid has been studied as a possible criterion of the differences in viscosity. Tryller (17) expressed the ratio of cations to phosphorus in terms of equivalent bases per gram atom of phosphorus and found the ratio to vary from 1.19 to 1.85. A ratio of 1.67, he found, produced a neutral starch. Samec (11) stated that the titrable acidity allowable for potato starch is 6 cc of N/10 solution of sodium hydroxide per 100 g of starch. One of the authors (9) found that in a limited series of starches the least saturated of the potato and canna starches were the strongest, but the valence of the cation was taken to be the more dominant factor, since a strong starch can be produced if it is saturated with monovalent cations.

The entire problem of the relationship between the electrolytes and viscosity of the starch granule is thus in a very unsettled state.

There is a paucity of analytical data from which to draw conclusions. Most of the work thus far conducted has been of a fundamental nature, in which the properties of the specially purified fractions have been studied. The importance of starch in the industrial world warrants more intensive investigation of the relationship of the electrolytes of the starch granule to viscosity, this property being selected because of its very practical importance in most of its industrial uses.

In previous work at this station, the line of attack has been somewhat different from that of other investigations in that the properties of the whole or unbroken granule have been studied, whereas

in most work the starch granule has been broken down and studied as a heterogeneous mixture or fractionated into its components.

The investigation reported in this paper consisted primarily of the determination of the variation of the phosphoric acid and cations in starches of various origins, the nature of their bonding, by means of electrodialysis and salt replacement, and the possible significance of these results in practical industrial uses of starch, as well as in plant chemistry. Circumstances have forced the abandonment of this line of investigation, but the results are presented with the hope that some of the promising lines of investigation may be continued by other workers in this field.

METHODS

ASHING OF STARCH

It was found impossible to ash starch by direct ignition, the difficulty encountered being that the sample tended to fuse into a hard black mass upon loss of its constituent water. Subsequent and long-continued heating failed to produce a light-colored ash. The alcohol-and-glycerin ignition method employed in the ashing of wheat flour proved to be too difficult to control because of frothing. The wet digestion method, using concentrated sulphuric and nitric acids in a tall beaker, was found to be too tedious and the large amount of acids required gave rise to "blanks" which were excessively high.

The following method was finally adopted as being the most practicable:

Twenty grams of starch was placed in a 100-ml platinum dish, and 10 ml of sulphuric acid (specific gravity 1.84) was added. If charring failed to start after 2 or 3 minutes of stirring, the dish was heated slightly near one side, after which the reaction became exothermic and proceeded with a vigorous liberation of steam and sulphur dioxide. The stirring was continued during the reaction to prevent the formation of large gas pockets which might have caused the sample to overflow. After the reaction had ceased, the charred mass remained porous and ashed well at a dull red heat. The ash was taken up with hydrochloric acid and the silica dehydrated by the official method.

PREPARATION OF STARCH

The method used for locally prepared starches was the usual one of grinding or shredding the rootstock or tuber, followed by extraction of the starch from the pulp held on a fine screen or cheesecloth. The starch was purified by repeated stirring, settling, and decanting, ordinary tap water being used unless otherwise stated.³ The starches were air-dried over a hot plate at a temperature not exceeding 40° C.

Relative viscosity was determined by the method previously outlined (9), which consisted in bringing the starch into solution by means of hot water delivered from a pipette. The viscosity was measured in a Stormer viscosimeter and calculated as relative viscosity by reference to a standard curve.

Electrodialysis was carried out in a 3-compartment cell of the type described by Humfeld and Alben (5).

³ The tap water of the city of Honolulu is of excellent quality and seldom exceeds 5 grains per gallon of salts, which are chiefly the chlorides of sodium and calcium.

ANALYTICAL METHODS

Determination of phosphate was made by the Bell-Doisy colorimetric method (2).

Several methods for determining calcium were tried but were found unsuitable. Among these were Lyman's nephelometric (stearate) method (6), a micro method by Mazza and Rossi (7), and a tungstate colorimetric method by Astrug, Mousseron, and Bouissou (1). The method finally chosen was gravimetric, the calcium being precipitated in oxalic acid solution (4), filtered, dried at 100° C., and weighed as the oxalate (14, p. 91). This procedure offers two advantages over that of igniting the residue to calcium oxide: (1) It results in a much greater weight of material, which is of especial importance for the small amount of calcium present; and (2) it permits the whole procedure to be handled in sintered-glass crucibles. The method was tried out on a "known" solution which contained other electrolytes in amounts calculated to give it about the same composition as the ash of starch. Table 1 shows the results obtained.

TABLE 1.—*Estimation of small known quantities of calcium by weighing as the oxalate ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$) dried at 100° C.*

Ca present	Ca found	Error	Ca present	Ca found	Error
<i>Milligrams</i>	<i>Milligrams</i>	<i>Percent</i>	<i>Milligrams</i>	<i>Milligrams</i>	<i>Percent</i>
1.795	1.700	-5	1.339	1.289	-4
1.786	1.728	-3	.893	.932	+4
1.346	1.262	-6			

Magnesium was determined by the Bell-Doisy (18) method. Potassium was determined gravimetrically by the chloroplatinate method. Sodium was determined gravimetrically as sodium-zinc-uranyl acetate.

ASH CONSTITUENTS OF PRINCIPAL COMMERCIAL STARCHES

A series of commercial samples of starch were secured from a starch broker. These samples were selected to represent the normal run of starches as they are received by the starch broker from different sources. In addition, a number of laboratory samples of edible-canna and other starches were prepared.

A determination of the principal electrolyte constituents was made in the above series of starches. Table 2 gives the results of the analyses.

If we may consider as significant a single analysis of a given kind of starch, certain similarities and dissimilarities between the different starches are apparent. Arrowroot is notable for its extreme paucity of phosphate, which, together with a high calcium content, gives this starch such an abnormal excess of bases present as to indicate the presence of other anions probably existing as occluded salts. Its extremely low magnesium content again brings us to the conclusion that it is the most unusual of this group. Cassava, corn, sago, and sweetpotato starches are very low in phosphate as compared with either potato or canna starches. Rice starch is worthy of notice for its high content of magnesium, and a sodium content which is five times that of the next highest. Wheat starch is the only one of the first group in which the electrolyte content is comparable with that of potato starch.

TABLE 2.—Ash constituents (percentage) of principal commercial starches

Sample no.	Kind of starch	Source *	PO ₄	Ca	Mg	K	Na	Relative viscosity	Ratio total bases ^b PO ₄
13	Arrowroot	Commercial	0.015	0.080	0.002	0.012	0.009	-----	30.90
16	Cassava	do	.041	.011	.003	.010	.012	-----	3.66
15	Corn	do	.046	.017	.008	.012	.018	-----	5.34
17	Rice	do	.161	.010	.024	.025	.125	-----	5.07
19	Sago	do	.036	.018	.003	.034	.014	-----	6.90
43	Sweetpotato	Laboratory	.048	.006	.005	.017	.006	-----	2.76
12	Wheat	Commercial	.176	.027	.008	.036	.025	-----	2.19
9	Potato	Commercial, domestic	.185	.032	.008	.067	.008	0.72	2.22
57	do	do	.154	.015	.006	.023	.009	.72	1.38
11	do	Commercial, Dutch	.255	.026	.010	.019	.015	.85	1.23
86	do	do	.247	.010	.009	.061	.014	1.20	1.32
10	do	Commercial, German	.171	.047	.009	.023	.011	.90	2.31
14	do	do	.156	.049	.010	.019	.009	1.01	2.52
53	do	Commercial, unknown	.189	.043	.010	.025	.008	1.00	2.01
54	do	do	.221	.028	.013	.025	.025	1.16	1.83
55	do	do	.152	.020	.021	.013	.013	.81	2.28
66	do	Laboratory, upland-wet	.173	.017	.021	.025	.016	1.13	2.16
Average for potato			.190	.029	.012	.030	.013	.95	1.93
Maximum			.255	.049	.021	.067	.025	1.20	-----
Minimum			.152	.010	.006	.013	.008	.72	-----
4	Edible canna	Laboratory, lowland, irrigated	.128	.002	.019	.018	.019	.89	2.19
5	do	Laboratory, lowland, dry	.140	.014	.016	.010	.020	.88	2.10
20	do	Laboratory, upland	.147	.011	.022	.018	.024	1.08	2.49
52	do	Commercial	.137	.005	.014	.021	.010	1.00	1.65
63	do	Laboratory, upland, dry	.136	.010	.016	.019	.016	.92	2.10
64	do	Laboratory, lowland, dry	.112	.006	.014	.021	.010	.85	2.07
89	do	Laboratory, upland, wet	.137	.008	.015	.025	.022	1.23	2.31
Average for edible canna			.134	.008	.017	.019	.017	.98	2.13
Maximum			.147	.014	.022	.025	.024	.123	-----
Minimum			.112	.002	.014	.010	.010	.85	-----

* All "commercial" samples were secured through the courtesy of Morningstar, Nicol, Inc., New York, who submitted them as typical of commercial starches; all laboratory samples were prepared at the Hawaii Agricultural Experiment Station, Honolulu, Hawaii.

^b Total milliequivalents of bases divided by millimols of PO₄.

* This sample from Waimea Starch Co., Waimea, Hawaii.

A comparison of the average electrolyte content of potato and canna starches shows that potato contains more phosphate, considerably more potassium, and a great deal more calcium, but less magnesium and sodium than do the canna starches. The only constituent determined which shows anything like a constant or characteristic value is phosphate, all others vary between much wider limits. In this group of unrelated samples there is no apparent relation between viscosity and the percentage of any constituent determined.

EFFECT OF STORAGE OF EDIBLE-CANNA ROOTSTOCKS AND POTATO TUBERS UPON THE ELECTROLYTES OF THE EXTRACTED STARCHES

Previous work by one of the authors (9) has demonstrated that minute amounts of added electrolytes are able to change the physical properties, and also that these same properties are affected by the

interval that elapses between the digging of the edible-canna rootstocks and the extraction of the starch. It was therefore considered possible that the change occurring during storage of the rootstock is primarily an exchange of cations between sap and starch, which is responsible for the concurrent change in viscosity.

Table 3 gives the effect of storage of edible-canna rootstocks and potato tubers upon the electrolytes of the extracted starches.

TABLE 3.—*Effect of storage of edible-canna rootstocks and of potato tubers upon the electrolytes of the extracted starches, expressed in milliequivalents per 100 g of starch*

EDIBLE-CANNA STARCHES								
Sample no.	Time of storage	Phosphate (PO ₄)	Calcium (Ca)			Magnesium (Mg)		
			Total	Dialyzable	Proportion dialyzed	Total	Dialyzable	Proportion dialyzed
	Days				Percent			Percent
89.....	1	4.27	0.41	0.01	2.4	1.23	0.10	8.1
90.....	6	4.37	.85	.29	34.1	1.64	.36	22.0
91.....	15	4.93	.99	.28	28.3	1.80	.40	22.2
92.....	30	5.00	1.27	.57	44.9	2.05	.45	22.0

POTATO STARCHES								
66.....	1	5.40	0.85	0.59	69.4	1.72	0.55	32.0
67.....	5	4.77	1.30	.34	26.2	1.89	.39	20.6
68.....	17	5.36	1.06	.50	47.2	1.89	.30	15.9
70.....	80	4.96	1.10	1.03	93.6	1.97	.18	9.1

EDIBLE-CANNA STARCHES									
Sample no.	Time of storage	Potassium (K)			Sodium			pH of starch	Relative viscosity
		Total	Dialyzable	Proportion dialyzed	Total	Dialyzable	Proportion dialyzed		
	Days			Percent			Percent		
89.....	1	0.64	0.64	100	0.90	0.46	46.4	0.91	1.23
90.....	6	.15	.13	86.6	.74	.42	56.7	.46	1.09
91.....	15	.15	.12	80.0	.65	.44	67.7	.45	1.05
92.....	30	.23	.11	47.8	.65	.44	67.7	.35	.95

POTATO STARCHES									
66.....	1	0.64	0.08	12.5	0.70	0.39	55.7	0.29	6.98
67.....	5	.49	.09	18.4	.52	.39	75.0	.40	1.35
68.....	17	.56	.09	16.1	.48	.35	72.9	.36	1.27
70.....	80	.67	.08	11.9	.61	.30	49.2	.24	1.29

In the canna series several changes occurred that were due to storage. The increase in phosphate was sufficiently large and regular to appear significant. The loss of monovalent cations was nearly equal to the increase in calcium and magnesium. The rate of change was greatest between the 1-day and 6-day storage period, the relative viscosity being reduced from 1.23 to 1.09. The abruptness of the initial drop might be taken as a strong indication that it was caused by the shifting of electrolytes toward a new equilibrium.

The potato series showed significant changes in total electrolytes and a remarkable increase in viscosity between the 1-day and the 5-day storage period. Large as the increase was, there is no reason why it should be assumed to be at its maximum after 5 days, since only four storage periods were investigated. Although the change in electrolytes indicates that the same forces were present as in the canna series, the magnitude of the change produced was less.

Inasmuch as the viscosity increased in spite of the increase in divalent and decrease in monovalent cations, it may be safely assumed that some other very potent force was present. If the amounts of cations dialyzed are considered as representing the active portion of the bases present, the members of the canna series exhibited toward viscosity approximately the same relationship as did total cations. By this method, however, the members of the potato series are brought into much better agreement with their respective viscosities. Sample 70 may safely be omitted in view of the fact that many of the tubers were rotting at the time the starch was extracted.

The considerable differences between the two series in the extent of replaceability of the various cations are shown in the column "proportion dialyzed." In view of the dissimilar natures of the canna- and potato-storage organs, it is remarkable that in the case of both magnesium and sodium, the proportions dialyzed have so nearly the same limits of variation in the two series.

The ratio of monovalent to total dialyzable cations exhibits toward viscosity an acceptable correlation within an individual series.

In these starches viscosity does not appear to be a function of hydrogen-ion concentration. In this study of active bases, uncooked whole granules were used, and every effort was made to preserve their original structure. The discontinuance of this project prevented a similar investigation of the active bases in a starch which had been thoroughly disintegrated as in a ball mill.

REPLACEMENT OF BASES BY SALTS OF VARIOUS CONCENTRATIONS

In view of the present importance attached to the relative replaceability of cations in various colloids, it was considered desirable to determine the replaceability of cations in the starch granule. Accordingly, starch samples were shaken with several concentrations of ammonium acetate and sodium chloride.

Table 4 gives the amounts of electrolytes removed from edible-canna starch no. 52 by distilled water, ammonium acetate, and sodium chloride.

For the sake of comparison, the total composition of edible-canna starch no. 52 and the amounts of the cations removed by electro-dialysis were included in this table. The treatment consisted in adding 750 ml of the reagent to 100 g of starch and agitating for 4 hours in an end-over-end shaker. After the starch had settled, the liquid was poured off and twice replaced with 150-ml portions of fresh reagent, shaken up, settled, and decanted. The combined liquids so obtained were freed from starch and analyzed. One cannot fail to be impressed by the magnitude of the amounts removed by distilled water alone. This loss of bases to distilled water had previously been observed in the lowering of the hydrogen-ion concentration produced by successive washings (9, p. 37). Since all these

starches were washed during the process of their preparation, it is not known whether these percentages represent additional amounts resulting from successive washings or whether more electrolytes are released by the intermediate drying of the starch. The ease with which even very dilute solutions of salts may cause an exchange of cations between the starch and the solution has been recognized in connection with the necessary use of pure water in the commercial preparation of starch (9, p. 42).

TABLE 4.—*Electrolytes removed from edible-canna starch no. 52 by distilled water, by solutions of ammonium acetate and sodium chloride of various strengths, and by electro dialysis*

[Results expressed as percentage of starch]

Treatment of starch	Strength of reagent	Phosphatc (PO ₄)	Calcium (Ca)	Magnesium (Mg)	Potassium (K)	Sodium (Na)
None		0.137	0.0048	0.0140	0.0210	0.0097
Distilled water		(a)	.0005	.0003	.0070	.0020
	0.001 E ^b	.014	.0007	.0012	.0145	.0038
	0.01 E	.017	.0014	.0035	.0185	.0053
Ammonium acetate	0.1 E	.012	.0016	.0110	.0140	.0045
	E	.007	.0022	.0056	.0117	.0064
	0.001 E	(a)	.0031	.0012	.0166	(a)
	0.01 E	(a)	.0048	.0031	.0210	(a)
Sodium chloride	0.1 E	(a)	.0048	.0061	.0202	(a)
	E	(a)	.0033	.0078	.0210	(a)
Electrodialyzed		(a)	.0003	.0018	.0210	.0038

^a Not determined.

^b "E," equivalent weight.

Ammonium acetate is evidently less effective than sodium chloride in the removal of cations. The most effective concentration is not the same for the two reagents, nor is it the same for all cations.

Salt replacement seems to be more effective than cataphoresis in removing cations from starch, with the one exception of potassium which is completely removed by either method.

RATE OF REMOVAL OF ELECTROLYTES BY CATAPHORESIS

As previously noted, electro dialysis was carried out in a 3-compartment cell, so constructed as to enable the removal of the contents of either chamber. This was done at various intervals and the contents titrated with acid or base to determine the rate and progress of the reaction. Figure 1 shows the rate and extent of removal of electrolyte from edible-canna starch by cataphoresis.

Nearly 80 percent as much base was removed in the first 10 minutes as in a total of 220 minutes. After 1 hour, the rate of removal dropped to a very low and fairly constant rate. The acid ions were removed at a nearly constant rate, decreasing only slightly toward the end. The total amount of anions removed in the 220-minute period was only about 60 percent of the amount of cations removed. Obviously, for any shorter interval, this proportion would be smaller. All the starches exhibited some inclination to migrate toward the anode, and some of them adhered strongly to that membrane, necessitating continuous scraping and stirring.

EFFECT OF VARIATION OF ELECTROLYTES IN PLANT SAP UPON THE STARCH IN VIVO

It is a well-known fact that the composition of the sap of a plant can be greatly changed by variations in the nutrient solution. An excess of one cation, potash, for example, sometimes represses the absorption of the other cations. Since in vitro one cation readily replaces another in the starch granule, the possibility suggests itself of thus changing the cations of the starch grain in vivo.

Edible-canna plants were grown in a series of water cultures in which the calcium and potassium were varied within wide limits while the other ions were kept constant. In one culture, no. 96-6, the concentration of the entire nutrient solution was made one fifth that of no. 96-3. The edible canna is not well adapted to water cultures, and considerable difficulty was experienced in maintaining growth.

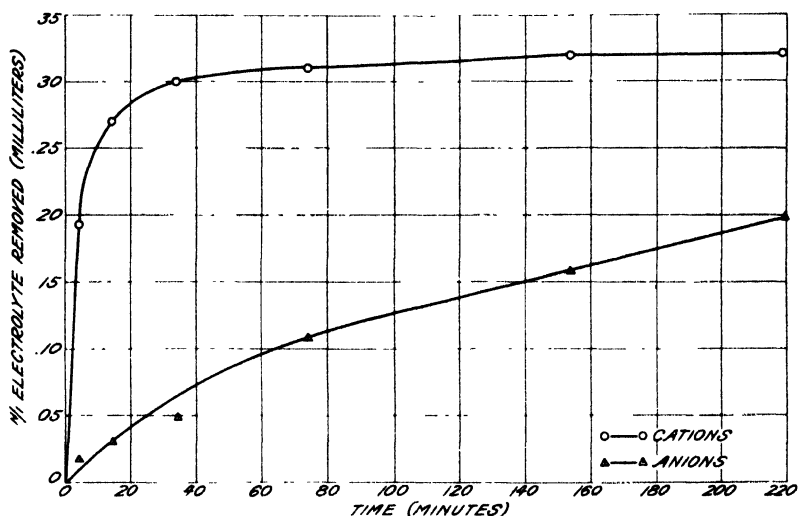


FIGURE 1.—Rate and extent of removal of electrolytes from a 50-g sample of edible-canna starch no. 52 by cataphoresis.

Enough tuber growth was made to secure a small sample of starch from each. Distilled water was used in the washing and purification of the samples. Table 5 gives the composition of the four starches with respect to phosphorus, calcium, and potassium.

TABLE 5.—Relation of composition of nutrient solution to that of starch from edible canna grown therein

Sample no.	Concentration of ions in nutrient solution			Concentration of ions in starch		
	PO ₄	Ca	K	PO ₄	Ca	K
	P.p.m.	P.p.m.	P.p.m.	Percent	Percent	Percent
96-1	475	391	10	0.087	0.0055	0.005
96-3	475	200	195	.056	.0055	.003
96-5	475	10	381	.056	.0034	.007
96-6	95	40	39	.055	.0041	.006

The results show no tendency on the part of the starch to be affected by the composition of the nutrient solution. The abnormality of the growth is shown in the small percentages of all three ions.

Table 6 gives the difference in electrolytes of starch from edible-canna tubers of a single hill, divided into four groups in different stages of development. A hill of edible canna grows more or less continuously, new rootstocks developing from axillary buds so that at any given time after about 9 months of growth there exist in the same hill rootstocks in all stages of development.

TABLE 6.—*Electrolytes in starch from rootstocks in different stages of development within a single hill of edible canna*

Sample no.	State of development of rootstock	Percentage of indicated electrolyte in the starch				
		PO ₄	Ca	Mg	K	Na
71	Group 1, dormant, stalk entirely dead.....	0.155	0.0212	0.021	0.012	0.014
72	Group 2, mature, stalk full grown.....	.156	.0206	.022	.009	.014
73	Group 3a, immature, stalk half grown.....	.199	.0329	.027	.035	.010
74	Group 3b, immature, rapidly developing spikelet....	.186	.0240	.028	.032	.010

In general, group 3a gave the highest concentration of electrolytes, potassium showing the greatest relative change. However, considering the fact that the rootstocks in group 1 were about 9 months old, while those in group 3 were just developing, the differences are not great.

Table 7 gives the electrolytes in starch and sap from edible-canna rootstocks stored for varying lengths of time. The sap represents the portion that could be expressed from the pulp by hand, after the tubers had been ground in a meat chopper preparatory to extracting the starch. For the sake of comparison, analyses of the corresponding starches are given.

TABLE 7.—*Electrolytes in starch and sap from edible canna rootstocks stored for varying lengths of time*

Sample no.	Time of storage of rootstocks	Material	Percentage of indicated electrolyte in the starch				
			PO ₄	Ca	Mg	K	Na
89	1	Starch.....	.0137	0.0082	0.015	0.025	0.0223
		Sap.....	.204	.0044	.033	.583	.0030
90	6	Starch.....	.140	.0171	.020	.006	.0170
		Sap.....	.239	.0036	.044	.478	.0023
91	15	Starch.....	.158	.0109	.022	.006	.0150
		Sap.....	.255	.0048	.050	.671	.0025
92	30	Starch.....	.160	.0254	.025	.009	.0149
		Sap.....	.337	.0023	.060	.743	.0026

Table 7 gives strong evidence that there is no direct chemical equilibrium between the plant sap and the ions combined with the starch. Not only is there no correlation between the changes in electrolytes during storage in starch and sap, but the ratio between the percentage of ions in starch and sap for the different ions is a vastly different one. For instance, in sample 89 the starch holds about 8 times as much

sodium as the sap, while the sap holds about 20 times as much potassium as the starch. Treatment of an extracted starch with a salt solution in vitro shows little specificity of cations, an excess of one cation being able to largely replace the other cations (11). The very large excess of potassium over the other cations in the sap would be expected to result in far more potassium in the starch than is the case. The greater concentration of calcium and sodium in the starch than in the sap suggests that these ions may be held in a relatively unionized condition. The same may be said of the large increase in calcium with a roughly constant calcium content of the sap. The percentage of the different ions in the starch might thus represent the cumulative effect of various changes in sap concentration during growth or storage, which fact would obscure any relationship between starch and sap at any given time.

While it is evident that the conditions of the foregoing experiments preclude exact and detailed conclusions, they furnish strong evidence that changes in starch electrolytes in vivo are small compared with the effect of similar electrolyte concentrations in vitro. Ionic absorption appears specific and not controlled by the relative concentration of the different ions in the sap. Such selectivity is characteristic of absorption of ions by the living plant cell. The present concept of starch as made up of a carbohydrate, protein, fatty acid, mineral complex adds to the evidence that starch is directly combined with, and affected by, the living protoplasm of the cell.

The extent of the variation in electrolytes caused by differences in climate may be judged by the maximum and minimum values for the different ions given in table 2 for edible canna. Rapid meristematic growth, as compared with dormancy in the same hill (table 6), produces a certain increase in electrolytes. The electrolyte changes taking place during germination (table 3) are of a different order from those of growth, i.e., rapid displacement of potassium, and equally rapid and continuous absorption of calcium to a point where potassium is much lower than the minimum for normal canna starches (table 2), and calcium is far in excess.

Table 8 gives the relative proportions of the ash constituents of the edible-canna tuber present in the starch and nonstarch portion of the tuber.

TABLE 8. --Proportion of ions held by the starch and nonstarch parts of the edible-canna tuber

Ion	Edible-canna tuber ^a	Extracted starch	Ions in starch expressed as proportion of tuber ^b	Proportion of ion in starch of ion in whole tuber ^c
	Percent	Percent	Percent	Percent
PO ₄	0.646	0.074	0.059	9.1
Ca.	.0343	.0027	.0022	6.4
Mg.	.205	.012	.010	4.9
K.	2.288	.019	.015	.7
Na.	.0620	.0092	.0074	9.0

^a Air-dry basis.

^b Column 3 by 80 percent because starch in the oven-dry edible-canna tuber runs about 80 percent.

^c Data in column 4 divided by data in column 2.

These data show the importance of starch as an inorganic storage material. Particularly is this true of phosphorus which is needed in large amounts during germination and in the very early development of the young plant. Decomposition of the starch granule would bring these mineral constituents into solution, possibly in organic combinations especially needed for early growth. For example, Russell (10) states that oxidation of carbohydrates involves the simultaneous formation of a hexose phosphate and probably explains the large amount of phosphorus compounds stored up in the seed.

SUMMARY

The major ash constituents were determined for a series of the principal commercial starches.

Edible-canna rootstocks and potato tubers were stored for various lengths of time, and the starch was extracted. The viscosity and composition of these starches were then determined. With the edible-canna series it was possible to show that the drop in viscosity was correlated with a drop in potassium and an increase in calcium. The same changes in electrolytes could not be established in the potato series. In view of the contradictory nature of these results, it was concluded that some method of evaluating the ions according to their activity might give more concordant results. For this purpose electrodialysis and salt-replacement methods were used. Electrodialysis did not change the relationship for the canna series but did assist in bringing the potato series into better agreement.

Salt replacement appears to be more effective than cataphoresis in the removal of bases, but less reliable in the matter of obtaining duplicates.

Significant amounts of cations are removable by shaking with distilled water.

Study was made of the effect of the changes in the electrolytes in plant sap on the starch *in vivo*. The results showed that while the electrolytes held by the starch are affected to some extent by differences in plant sap and growth conditions, the effect is small compared with the corresponding effect on the starch *in vitro*. Electrolyte changes in starch due to storage of the rootstocks are larger than those produced during normal growth.

It was concluded the electrolytes occurring in starch *in vivo* are largely determined by the protoplasm of the plant cell rather than by direct chemical equilibrium with the electrolytes of the plant sap.

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STUDIES ON PHYSIOLOGIC SPECIALIZATION IN *TILLETIA TRITICI* AND *T. LEVIS* IN THE PACIFIC NORTHWEST¹

By H. H. FLOR²

Associate pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Bunt, or stinking smut, has long been the most important disease of wheat (*Triticum vulgare* Vill.)³ in the Pacific Northwest. In the United States it is caused by two species of fungi—namely, *Tilletia levis* Kühn, whose spore wall is smooth, and *T. tritici* (Bjerk.) Wint., whose spore wall is reticulated or rough. Despite the development of resistant varieties and of improved methods of seed treatment, losses due to bunt have increased in recent years, not only in the Pacific Northwest but also in the regions of the Middle West⁴ where hard red spring and winter wheats are grown. The increased economic importance of bunt has stimulated the study of this disease by research workers in various parts of the country. Their investigations have shown that one of the major contributory factors in the prevalence of bunt has been the appearance of new physiologic forms of the bunt fungi.

The objects of the investigation reported herein were: (1) To determine the prevalence and distribution of the two species of bunt fungi, *Tilletia levis* and *T. tritici*, in the Pacific Northwest; (2) to determine the prevalence and distribution of the physiologic forms of these bunt fungi and the relation between the varieties of wheat and distribution of the physiologic forms; (3) to ascertain whether pathogenicity of the physiologic forms was constant or was easily modified; and (4) to ascertain what correlation, if any, existed between the morphological and cultural characteristics and the pathogenicity of the physiologic forms.

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³ According to the rules of botanical nomenclature the name of this species is *Triticum aestivum* L.; but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that form.

⁴ BOERNER, E. G., HASKELL, R. J., and MEIER, F. C. SMUTTY WHEAT. U.S. Dept. Agr., Rpt. no. 11, 5 p. 1930. [Mimeographed.]

REVIEW OF LITERATURE

Stakman (17) ⁵ has stated that physiologic forms of fungi may be differentiated by (1) pathogenic effects on different species and varieties of host plants, (2) morphological characteristics, (3) cultural characteristics on artificial media, and (4) physicochemical reactions. The economic importance of demonstrating the existence of physiologic specialization of bunt is concerned primarily with pathogenicity. Consequently, most of the investigations consisted of testing the resistance and susceptibility of a number of wheat varieties to bunt collections.

Faris (3), in 1924, was the first to present evidence indicating that physiologic specialization occurred in *Tilletia levis* and *T. tritici*. Later, Rodenhiser and Stakman (14), Reed (11), Roemer (15), Gaines (5), Weston (18), Bressman (2), Holton (7), Aamodt (1), and others demonstrated physiologic specialization of the bunt organisms by testing the pathogenicity of a number of collections to selected varieties.

Although a major portion of the work on physiologic specialization of bunt has consisted of pathogenicity tests, other means of differentiating the forms have been studied. Bressman (2) reported that collections had different optimum temperatures for spore germination. He also differentiated one form by the occurrence of masses of bunt spores on the outside of the glumes of the wheat head. Kienholz and Heald (9) found that chlamydospore mass cultures of Gaines' ⁶ forms differed greatly in cultural characteristics on various solid media. They found, however, that these cultural characteristics were not constant. Rodenhiser (13) has shown that forms differ in respect to the stunting effect on the culms of the host plants.

MATERIALS AND METHODS

Bunt collections were made from fields in the major wheat-growing sections of Oregon, Washington, and northern Idaho in 1929 and 1930. Where bunt was abundant, as in most fields, the collections were composed of 50 or more bunted heads taken at random from as many plants. In a few fields bunt was so scarce that smaller collections had to suffice. Spores from one or more bunt balls from each head were examined under a microscope in order to determine the proportion of *Tilletia levis* and *T. tritici*. In this manner the heads of each collection were separated according to the species of bunt. Bunt balls from the heads thus separated were ground and used as inoculum for the varietal host testers in order to determine the range of pathogenicity of each collection. Although 60 percent of the collections contained both *T. levis* and *T. tritici*, the occurrence of both species in a single head was rare. Examination of the ground bunt used as inoculum disclosed the fact that in only 7 of the 240 samples were both the rough-spored and smooth-spored species present.

The collections made in 1929 were propagated on Jenkin club spring wheat at Pullman, Wash., in 1930. Unfortunately, part of the soil in the increase plot had been treated with a weed killer and the wheat in that area died. A number of the collections, therefore, were lost. The ranges of pathogenicity of the surviving collections and of collections made in 1930 were determined in 1930-31 by inoculation of differential varieties.

⁵ Reference is made by number (italic) to Literature Cited, p. 212.

⁶ GAINES, E. F. WHEAT SMUT HAS BEEN INCREASING. U. S. Dept. Agr., Off. Coop. Ext. Work, Ext. Path. 6(2):14-15. February 1928. [Mimeographed.]

GEOGRAPHIC DISTRIBUTION

Tilletia tritici, the rough-spored species, has long been the predominant one in the Pacific Northwest, and for a long time it was considered to be the only species present there. Kienholz and Heald (9) reported that *T. levis*, the smooth-spored species, was found in only 2 of 631 wheat fields examined in 1919 in eastern Washington. However, surveys made by them in 1927 and 1928 showed that *T. levis* was present in all the principal wheat-growing districts of Washington, although *T. tritici* continued to be the predominating species.

The distribution of *Tilletia levis* and *T. tritici* in the Pacific Northwest, as determined from the 1929 and 1930 collections, is shown in



FIGURE 1.—Map showing distribution of *Tilletia levis* and *T. tritici* in Washington, Oregon, and northern Idaho and approximate proportions of each species in field collections made in 1929 and 1930 are indicated as follows:

- = Field collection containing 100 percent *T. tritici* and 0 percent *T. levis*;
- ▲ = Field collection containing 90-99 percent *T. tritici* and 1-10 percent *T. levis*;
- = Field collection containing 50-89 percent *T. tritici* and 11-49 percent *T. levis*;
- △ = Field collection containing 11-49 percent *T. tritici* and 50-89 percent *T. levis*;
- = Field collection containing 1-10 percent *T. tritici* and 90-99 percent *T. levis*.

figure 1. Although 60 percent of the field collections contained both species, *T. tritici* continued to predominate. This species was found in every field examined, whereas *T. levis* was found in only 60 percent of the fields, and a summation of all the heads examined showed that 81.2 percent were smutted by *T. tritici*. West of the Cascade Mountains, *T. levis* predominated in 8 of the 10 fields examined. East of the Cascades, *T. levis* was most abundant on Albit and Ridit wheats in the extreme eastern part of Washington, on Turkey in both Washington and Oregon, and, regardless of the variety, in fields in the Grande Ronde Valley northeast of La Grande, Oreg., and in the Camas Prairie area southeast of Lewiston, Idaho. In the district extending from

Dayton, Wash., to Heppner, Oreg., very little *T. levis* was found. The wheat varieties chiefly grown there were susceptible to all the known forms of bunt, yet most of the collections contained *T. tritici* alone and none contained more than 10 percent of *T. levis*.

PHYSIOLOGIC FORMS

Previous investigators (1, 2, 5, 7, 8, 11, 12, 14, 15, 18) used field collections as the basis for determining physiologic forms of the bunt fungi. Holton (7) has pointed out, however, that from what is known of the genetics of the smuts, it is doubtful whether the term "form" can properly be applied to these field collections. The writer (4) found that monosporidial lines were haploid and that hybridization readily occurred between the two species, as well as between various so-called "physiologic forms." Although the occurrence of hybridization in nature has not been demonstrated, there appears to be ample opportunity for it to take place in view of the fact that relatively small samples taken at random from fields in the Pacific Northwest showed that 60 percent of the fields contained the two species. The possibility of hybridization is further increased by the presence in this region of several pathogenically distinct strains of bunt (2, 6). The presence of the two species of *Tilletia* in a majority of the fields and the known occurrence of a number of pathogenic strains make it probable that a collection would contain a mixture of forms even if hybridization were not a factor. Furthermore, it must be recognized that nothing is known in regard to the intergrading of the forms and that it is possible to distinguish only those that have very distinct pathogenic properties. Consequently, an attempt to classify collections according to forms can, at best, be but a grouping of those collections possessing similar ranges of pathogenicity, as shown by the percentage of bunted heads produced on each of a limited number of wheat varieties that serve as differential hosts. From the standpoint of simplicity and utility it seems best, for the present at least, to continue to designate those collections having different pathogenic properties as "physiologic forms," although "form groups" probably would be a more accurate designation.

The results of some of the more outstanding pathogenicity tests with collections of *Tilletia levis* and *T. tritici* are shown in table 1.

Although these collections may have contained a mixture of forms, the results were sufficiently distinct to justify an attempt at classification. Forms T-1, T-2, and T-3 possessed pathogenic properties similar to Gaines'⁷ forms of the same numbers. Form T-1 was the old *Tilletia tritici* form to which all the differential varieties were resistant, except Hybrid 128, which was susceptible to all forms. Form T-2 was very virulent, attacking Turkey (C.I. 6175), Albit, Hussar, and White Odessa. Form T-3 differed from form T-2 in that it attacked Albit and White Odessa but not Turkey (C.I. 6175) or Hussar. Form T-4 differed from form T-3 in that it attacked Turkey (C.I. 6175) but not Albit. The examples chosen to illustrate forms T-5, T-6, and T-7 make them appear to be distinct. Results from pathogenicity tests of other collections might have been chosen, however, that would have shown a gradual gradation from form T-1 to these three groups. The production of 7.6 and 66.3 percent smut

⁷ GAINES, E. F. Op. cit.

in Turkey (C.I. 6175) by forms T-1 and T-5, respectively, undoubtedly justified the separation of these forms. Likewise a separation of forms T-6 and T-7 from form T-5 appeared to be justified by the production of 18.5 percent of smut in Oro by form T-6 and 22.2 percent in Hohenheimer by form T-7. However, as all gradations of infection occurred between these extreme examples, the propriety of setting up an arbitrary percentage number separating the forms is questionable. It is possible that the inoculum obtained from these collections contained a mixture of forms that may be separated by a process of varietal screening. It is also possible that in some varieties there is a complex interrelationship between host resistance and pathogenicity of the fungi and that in these cases it may be impossible to purify a collection for specific host pathogenicity. On the other hand, it appears that some varieties are either resistant or susceptible to certain collections. For example, the susceptibility of Albit to 150 collections of *T. tritici* was determined. Of these collections 133 produced less than 5 percent of bunted heads and 17 produced more than 21 percent of bunted heads.

TABLE 1.—Physiologic forms of *Tilletia tritici* and *T. levis* in representative field collections made in the Pacific Northwest in 1929 and 1930, as shown by results of inoculations of 9 differential varieties of wheat at Pullman, Wash., 1931

Species	Form no	Collection no	Percentage of bunted heads in—								
			Hybrid 128 (C.I. no. 4612)	Turkey (C.I. no. 6175)	Ridit (C.I. no. 6703)	Oro (C.I. no. 8220)	Hohenheimer (C.I. no. 11459)	Turkey selection (C.I. no. 7366)	Albit (C.I. no. 8275)	Hussar (C.I. no. 4843)	White Odessa (C.I. no. 4655)
<i>Tilletia tritici</i>	T-1	A-152	86.8	7.6	0.8	0.4	1.5	1.0	0.5	0	1.6
	T-2	B-108	87.5	60.0	8.7	.2	1.5	0	40.8	28.5	66.7
	T-3	A-155	88.2	4.4	0	0	4.0	1.8	34.8	.2	39.8
	T-4	C-101	90.4	40.9	2.7	0	1.4	0	3.8	0	43.3
	T-5	B-112	84.0	66.3	1.5	4	1.4	0	.2	0	2.4
	T-6	C-2	39.1	72.0	1.0	18.5	0	2.3	0	0	0
	T-7	C-103	90.5	74.0	.7	2.1	22.2	0	.8	0	1.0
<i>Tilletia levis</i>	L-1	B-107	85.1	87.0	0	3.7	1.4	1.2	0	0	.4
	L-2	A-161	89.1	98.0	8.7	4.5	1.4	1.5	96.2	74.6	90.7
	L-3	C-103	77.1	50.0	26.6	7	1.5	2.2	1.1	0	1.5
	L-4	A-162	90.0	93.2	.7	20.4	0	3.1	90.3	42.2	70.6
	L-5	A-42	88.1	82.9	1.8	3.0	13.6	3	6.3	.2	11.3
	L-6	A-107	88.6	15.6	1.9	2.7	3.3	12.8	3.2	0	3.6

* C.I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

The inoculation tests with the collections of *Tilletia levis* showed the existence of only two sharply differentiated groups as illustrated by forms L-1 and L-2. Albit, Hussar, and White Odessa were resistant to form L-1 and susceptible to form L-2. Form L-3 differed from form L-1 in that it attacked Ridit. Numerous gradations in percentage of bunt in Ridit were obtained, from 0 percent in the type collection of form L-1 to 26.6 in that of L-3. However, since Weston (18) and Bressman (2) have shown that Ridit apparently screens out of a collection those forms to which it is susceptible, and since Ridit was very resistant to most of the collections falling in form group L-1, it was arbitrarily decided to place in a new group those collections producing more than 15 percent of bunt in Ridit. The screening effect of Ridit was illustrated by a bunted Ridit plant which was

found in a field of Albit. Inoculum from this plant produced 40.9 percent of bunt in Ridit, while that obtained from Albit plants in the same field produced 11.4 percent of bunt in Ridit. Form L-4 was separated from form L-2 by the virulence of the former to Oro, and the virulence of form L-5 to Hohenheimer separated it from form L-1. Form L-6 was differentiated by the reaction of the two strains of Turkey used in these trials. Both strains were moderately resistant to this form, whereas one (C.I. 6175) was susceptible and the other (C.I. 7366) was very resistant to all the other forms of *T. levis*.

VARIETAL DISTRIBUTION

The relation between the varietal source of the collections and the species and physiologic forms of bunt is shown in table 2.

TABLE 2.—Relation between the variety of wheat grown and the species *Tilletia levis* and *T. tritici* and their physiologic forms contained in field collections made in the Pacific Northwest in 1929 and 1930

Wheat variety from which collection was made	Number of field collections containing—								
	Only <i>T. tritici</i>	<i>T. tritici</i> and <i>T. levis</i>	<i>T. tritici</i> forms—				<i>T. levis</i> forms—		
			T-1	T-2	T-3	T-4	L-1	L-2	L-3
Albit.....	1	13	0	1	9	0	1	9	1
Baart.....	7	12	10	0	0	0	7	1	0
Pacific Bluestem.....	1	3	4	0	0	0	3	0	0
Coppel.....	1	1	1	0	0	0	1	0	0
Dicklow.....	2	1	3	0	0	0	1	0	0
Federation.....	14	3	16	0	0	0	3	0	0
Goldcoin (Fortyfold).....	3	12	13	1	1	0	8	3	0
Hybrid 123.....	0	1	0	0	0	0	0	0	0
Hybrid 128.....	16	11	24	0	0	0	6	0	0
Hybrid 143.....	0	1	1	0	0	0	0	0	1
Jenkin.....	7	4	9	0	0	0	3	0	0
Marquis.....	0	1	1	0	0	0	1	0	0
Mosida.....	1	3	2	0	2	0	3	0	0
Red Russian.....	1	2	1	0	0	0	2	0	0
Ridit.....	0	5	4	0	0	0	1	1	2
Sol (Sun).....	1	1	0	0	1	0	0	1	0
Pilcrow.....	1	0	1	0	0	0	0	0	0
Triplet.....	11	9	17	1	0	0	8	0	0
Turkey.....	2	19	17	0	0	0	15	1	1
Mixture and unidentified.....	4	7	9	0	0	1	5	0	1
Total.....	73	109	133	3	13	1	68	16	6

Tilletia levis was present in most of the fields of Albit, Ridit, and Turkey varieties resistant to the old form of *T. tritici*. The high percentage of species mixtures in fields of the susceptible Goldcoin (Fortyfold) was due to the fact that this variety was extensively grown in districts where *T. levis* was abundant, particularly in regions west of the Cascades, in the Grande Ronde Valley of Oregon, and in the Camas Prairie region of Idaho. A number of fields of Hybrid 128 and Triplet in these districts also contained both species. *T. tritici* proved to be the prevailing species throughout the wheat-growing section from eastern Washington to north-central Oregon, where bunt-susceptible varieties were the principal ones grown.

It has already been pointed out that a majority of the field collections were mixtures of the two species and that probably many of them also were mixtures of physiologic forms. Consequently, an accurate determination of all the physiologic forms present in each collection could not be made in 1 year. Since the primary object of

the work was to determine the prevalence and distribution of the new physiologic forms, it was decided to dispense with borderline groups and to include in the classification only those forms which were undoubtedly distinct. Therefore those collections that may have contained the borderline forms T-5, T-6, and T-7 of *Tilletia tritici* were grouped with form T-1, and those containing form L-4 and forms L-5 and L-6 of *T. levis* were grouped with forms L-2 and L-1, respectively.

The largest proportion of the collections (84 percent) belonged in the groups containing forms T-1 and L-1. These were the forms of *Tilletia tritici* and *T. levis* to which Redit, Albit, Hussar, and White Odessa were resistant. Most of the collections placed in these groups attacked Oro, Hohenheimer, and Turkey selection (C.I. 7366) very weakly, while many produced only a small percentage of bunt in Turkey (C.I. 6175).

In the region surveyed in 1929 and 1930, Albit, Redit, and Turkey (C.I. 6175) were the only resistant wheats grown commercially to any extent. Albit was the best differential variety, and 20 of the 39 collections containing the so-called new forms were obtained from fields of this variety. The one collection of form L-1 from an Albit field was derived from a single bunted head that might have been a volunteer plant of Hybrid 128, a susceptible variety closely resembling Albit. When tested on Redit, the collections of *Tilletia tritici* were not sufficiently virulent to warrant the establishment of a new form. Three of the four collections of *T. levis* on Redit were strongly pathogenic. One of these belonged to form L-2, to which Albit, Hussar, and White Odessa were very susceptible and Redit moderately susceptible. The other two collections bunted Redit severely but did not attack the other resistant varieties. These collections were classified as form L-3. According to Gaines,⁸ Turkey (C.I. 6175) was resistant to the old form of *T. tritici* (form T-1), but was susceptible to the new form T-2 and to all forms of *T. levis*. In the present tests all the *T. tritici* collections obtained on Turkey were similar to Gaines' old form T-1, except that they attacked Turkey (C.I. 6175) normally. Tests with *T. tritici* collections from other varieties gave all gradations of infection on Turkey (C.I. 6175), from cases showing a small percentage, as Gaines found in the old form T-1, to cases in which Turkey was more severely bunted than Hybrid 128. Fifteen of the 17 collections of *T. levis* from Turkey contained forms belonging to group L-1. One collection, from Pomeroy, Wash., bunted Albit severely and was placed in group L-2; and another collection, from southwest of Colfax, Wash., attacked Redit but not Albit and was placed in group L-3.

Although most of the virulent forms found in this survey were collected from fields of resistant varieties, the exceptions are worthy of note. Form T-2, collection B-108, the most virulent of all the *Tilletia tritici* collections, was obtained at Pendleton, Oreg., from a field of Triplet wheat. The other *T. tritici* collections containing form T-2 were obtained from a field of Albit wheat south of Spokane, Wash., and from a field of Goldcoin (Fortyfold) in the Camas Prairie district southeast of Lewiston, Idaho. Form T-3 was obtained from 9 fields of Albit and 2 fields of Mosida in the vicinity of Pullman, Wash.; and west of the Cascades, from 1 field of Goldcoin (Fortyfold)

⁸ GAINES, E. F. Op. cit.

south of Portland, Oreg., and from 1 field of Sol (Sun) wheat north of Kalama, Wash. The one collection of form T-4 was obtained from an unidentified variety on the Camas Prairie of Idaho. Form L-2 of *T. levis* was obtained from 9 fields of Albit near Pullman, Wash.; from 1 field of Baart at Waterville, Wash.; from 3 fields of Goldcoin (Fortyfold) in the Grande Ronde Valley of Oregon; from 1 field of Turkey near Pomeroy, Wash.; and from the field of Sol (Sun) wheat north of Kalama, Wash. (west of the Cascades), in which form T-3 was found. Form L-3 was found only in eastern Washington on Albit, Ridit, and Turkey, and on the Camas Prairie of Idaho on Hybrid 143 and on an unidentified variety, probably selection C. This selection is one of Gaines' varieties that has never been officially released.

SOIL INFESTATION

Soil infestation is a major problem in the wheat-growing sections of the Pacific Northwest, east of the Cascade Range. In the fall of 1929 seed of Hybrid 128, treated with formaldehyde, was sown at Pendleton, Moro, and Corvallis, Oreg., and at Puyallup and Pullman, Wash. Since no bunt was produced at Corvallis or Puyallup, which are west of the Cascades and out of the usual region of soil infestation, it was evident that the seed was clean and that the bunted heads produced at Moro, Pendleton, and Pullman were due to soil infestation. Microscopic examination of the bunted heads and pathogenicity tests showed that only form T-1 of *Tilletia tritici* was present in these collections. Apparently *T. levis* and the more virulent strains of *T. tritici* had not become sufficiently abundant to be important factors in soil infestation.

ADAPTATION AND SCREENING

The question often has been raised as to whether or not the bunt fungi are able to change their virulence on association with resistant varieties so that they will be able to infect such varieties normally. Weston (18) and Bressman (2) have been able to increase the percentage of bunt in Ridit by inoculating seed of this variety with bunt obtained from bunted Ridit heads. Because of the limited knowledge concerning the genetics of the bunt fungi and the inability to know whether the collections of bunt contain one or more forms, it has been a debatable question as to whether this apparent change in virulence is due to the adaptation of the fungus to the host or to a screening-out process whereby only those forms in the collection are propagated that are able to attack the resistant host normally.

Through the courtesy of Gaines and Bressman, the writer was supplied with their collections. Gaines' form T-2 was his most virulent form of *Tilletia tritici* and had been increased on Hussar for several generations. Hussar was resistant to all other forms of *T. tritici* known to be present in the Pacific Northwest. Bressman's collection no. 28 was his most virulent form of *T. levis*, the only one attacking Oro severely. This collection was obtained in powdered form, and a microscopic examination showed that a small percentage of *T. tritici* was present, as had been noted by Bressman (2). This collection was increased on Hybrid 128 and on Oro, at Puyallup, Wash., in the season of 1929-30. Both varieties were severely bunted, but microscopic examinations showed that *T. levis* was the only species of bunt present on Oro, whereas some heads of Hybrid

128 contained *T. tritici* and others contained *T. levis*. However, the two species were not present in any single head. Nine differential varieties were inoculated with *T. levis* from Oro and with *T. tritici* from Hybrid 128, as well as with inoculum of Gaines' form T-2 obtained from six differential varieties. The results are shown in table 3.

TABLE 3.—Screening effect of host variety on percentages of bunt in the same variety and in certain other varieties in the subsequent crop

Differential varieties inoculated	C 1 no	Percentage of bunted heads of differential varieties inoculated with -							
		Bressman's no 28 of		Gaines' form T-2 of <i>Tilletia tritici</i> from -					
		<i>T. levis</i> from Oro	<i>T. tritici</i> from Hybrid 128	Hybrid 128	Ridit	Oro	Hohenheimer	Albit	Hussar
Hybrid 128	4512	75 0	77 5	35 9	38 6	32 7	15 3	68 8	49 4
Turkey	6175	84 2	87 0	15 7	55 6	75 8	68 9	82 2	73 3
Ridit	6703	5	2 2	2	5	.4	0	1 1	1
Oro	8220	58 2	2 2	1 2	(a)	1 6	1 4	2 2	2 6
Hohenheimer	11458	0	13 0	0	(a)	3 0	0	4 8	3 0
Turkey selection	7306	28 2	4	0	(a)	0	0	5	0
Albit	8275	0	83 4	26 2	14 9	22 1	7 7	18 5	37 8
Hussar	4843	0	57 2	22 7	24 2	35 3	17 0	27 4	24 3
White Odessa	4655	5	87 5	46 6	(a)	36 3	16 6	46 6	67 5

* Insufficient inoculum adequately to inoculate all varieties

There were conspicuous differences in pathogenicity of the *Tilletia levis* and *T. tritici* separated from Bressman's collection no. 28 by varietal screening. Oro was severely bunted by the strain of *T. levis* obtained from this collection, whereas Albit, Hussar, and White Odessa were highly resistant. On the other hand, Oro was resistant to the *T. tritici* obtained from this collection and Albit, Hussar, and White Odessa were susceptible.

The results obtained with Gaines' form T-2 indicated that its pathogenicity had not been changed by adaptation or by the screening effect of the host. A deficiency of smut from Ridit and Hohenheimer probably accounted for some of the variations, but a special effort was made to give each variety its maximum spore load with inoculum derived from that variety. Form T-2 had been propagated on its differential variety (Hussar) for several generations and there was no indication of adaptation or additional screening effect. The pathogenicity of this collection apparently had been purified to such an extent that it was not altered by being propagated on the other differential hosts used.

MORPHOLOGICAL CHARACTERS

During the examination of the spores to determine the species present in the collections, a number of heads were found which contained spores having distinct morphological characteristics. These spores differed in size, color, and character of reticulations. Some of the bunt balls were very hard and it was exceedingly difficult to powder them, even with a mortar and pestle, while other bunt balls were so soft that it was virtually impossible to shell them from the

head without rupturing the wall of the spore ball and allowing the enclosed spores to run out. Bunted heads containing these morphological types were separated from the collection proper and propagated on Jenkin wheat at Pullman, Wash., in the summer of 1930. An examination of the increased material showed that the distinguishing morphological characters had been retained.

The standard varietal host testers were inoculated with the increased material the following season, but no correlation was found between morphological characters and pathogenicity, with the possible exception that the selections having large spores with very pronounced reticulations produced little bunt even on susceptible Hybrid 128. The morphological characteristics of spores and spore balls were transferred to the succeeding generation, but the pathogenicity tests showed that all these variable selections belonged to form T-1.

DIFFERENTIAL EFFECT OF FIELD TEMPERATURES

The effect of temperature on percentage of bunt produced by five of Gaines' ⁹ physiologic forms was tested by sowing inoculated seed of a susceptible variety (Hybrid 128) and that of a resistant variety (Albit) in quadruplicate plots at weekly intervals from September 12 to November 11, 1930. The temperature during this series of sowings ranged from an average of 66° F. in the first week, which was too high for satisfactory bunt infection, to 35° in the last week, which was too low. This wide variation in temperature, however, produced no significant differences in the percentages of bunt among the forms tested.

CULTURAL STUDIES

Cultural studies are an important phase in an investigation of physiologic forms, not only for finding new forms but also because of the possibility of establishing a correlation between cultural and pathogenic characteristics. If it were possible to determine the pathogenicity of a bunt collection by its cultural characteristics a great deal of time would be saved, because cultural characteristics may be determined in the laboratory in a few weeks, whereas field pathogenicity tests require from one to several years.

The cultures used in these studies were obtained from Gaines' ¹⁰ physiologic forms T-1, T-2, T-3, L-4, L-5, L-6, and L-7. The writer has continued to apply the terms T-1, T-2, and T-3 to collections having pathogenic properties similar to those of Gaines' three physiologic forms of *Tilletia tritici*. Because of the recent extensive investigations of physiologic specialization in the bunt fungi, it appeared desirable to start a system of physiologic-form classification wherein forms of the two species would be completely separated. Consequently, those collections having the pathogenic properties of Gaines' form L-5 have been placed in the L-1 group. Pathogenicity tests showed that while Gaines' forms L-4, L-6, and L-7 may have possessed minor pathogenic differences, they were sufficiently alike to be placed in the L-2 group. Hence, in these cultural studies, the cultures derived from Gaines' forms L-4, L-6, and L-7 have been designated L-2-a, L-2-b, and L-2-c, respectively.

⁹ GAINES, E. F. Op. cit.

¹⁰ GAINES, E. F. Op. cit.

The method whereby single primary sporidial cultures of bunt were obtained has already been described (4). A number of media were tested in addition to those used by Sartoris (16) and Kienholz and Heald (9) in their studies on culturing the bunt fungi, but none was found to be superior to potato-dextrose agar, either for mycelial growth or for differentiating properties.

TYPES OF MYCELIUM IN CULTURES

Preliminary tests showed that the two distinct types of mycelial growth, with numerous variations, observed by Sartoris (16) and Kienholz and Heald (9) in chlamydospore mass cultures, were produced also by cultures derived from single sporidia. Microscopic examination revealed that the white cartilaginous heaped-up type was composed largely of fine mycelial threads and produced very few secondary sporidia in the early stages of formation and none later. The few sporidia produced by the white type of mycelium were found to reproduce only this type of mycelial growth. The gray flat type of mycelial growth was composed largely of mycelial threads that were much branched and twisted and were 3 to 4 times as thick as those of the white type. The gray mycelium produced an abundance of secondary sporidia from which the gray type of mycelium developed in the early stages of growth. Later the white type of growth was also produced in these cultures.

EFFECT OF TEMPERATURE ON MYCELIAL GROWTH

The effect of temperature on mycelial growth was determined by growing monosporidial cultures of Gaines' ¹¹ physiologic forms of bunt on potato agar with 2 percent dextrose in incubators at 7°, 12°, and 18° C., and at room temperature (20° to 25°). In general, cultures of all the forms responded similarly. At 7° growth was slow, and only the gray type of mycelium was produced; no chromogenesis of the medium occurred. At 12° and 18° growth was fairly rapid and both the gray and white types of mycelium were produced. At the lower temperature the gray type predominated, whereas at the higher temperature the two types were produced in about equal quantities, although considerable variation occurred between the individual cultures. At room temperature (20° to 25°) mycelial growth was slow and almost entirely of the white type. Chromogenesis was prominent at temperatures from 12° to 25°, except in cultures of form L-1.

EFFECT OF SUGAR CONTENT OF THE MEDIUM

Monosporidial cultures of six-physiologic forms of bunt were grown on potato agar containing 0, 0.5, 1.0, 2.0, 4.0, and 6.0 percent of dextrose, respectively, and incubated at 15° to 18° C. It was found that these percentages of sugar in the medium produced a gradation from the gray to the white type of mycelium similar to that produced by different temperatures. Although the individual cultures differed considerably in appearance, they showed the same general tendency in regard to chromogenesis and type of mycelium produced. The effect of the sugar (dextrose) content of the medium on chromogenesis and mycelial development of a monosporidial culture of

¹¹ GAINES, E. F. Op. cit.

Tilletia levis form L-2-b is shown in figure 2. Only the gray type of mycelium and no chromogenesis was produced on the plain potato agar. There was a tendency to produce the white type of mycelium at the

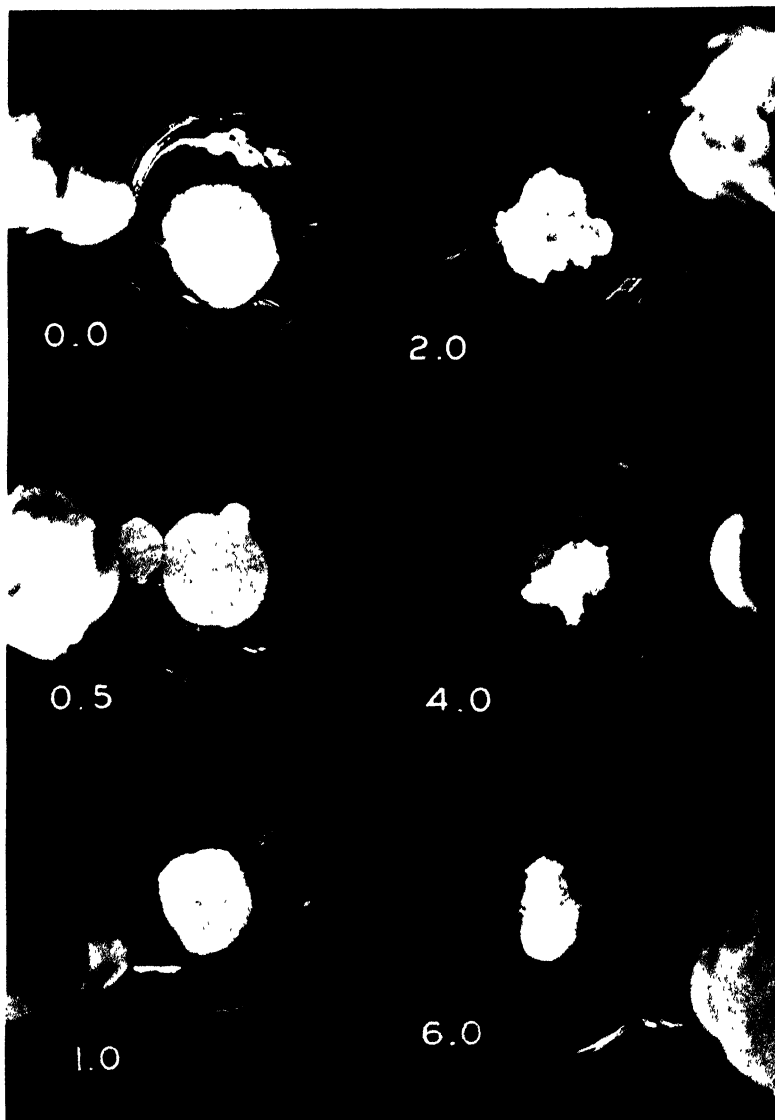


FIGURE 2 - Cultures derived from a single primary sporidium of *Tilletia levis* form L-2-b grown on potato agar containing 0, 0.5, 1.0, 2.0, 4.0, and 6.0 percent of dextrose, as indicated, and incubated at 15° to 18° C. The proportion of the white heaped-up mycelium to that of the gray flat type increased with the sugar content of the medium.

center of the colony grown on potato agar with 0.5 percent of dextrose. With each increase in the percentage of dextrose in the agar, there was an increase in the proportion of the white to the gray type of mycelium. The mycelium produced on the potato agar with 6 percent of dextrose

was entirely white except for a few marginal spots of gray. Cultures of Gaines' forms T-1, T-2, and T-3 of *T. tritici* and L-2-a and L-2-b of *T. levis* produced chromogenesis in all media to which dextrose had been

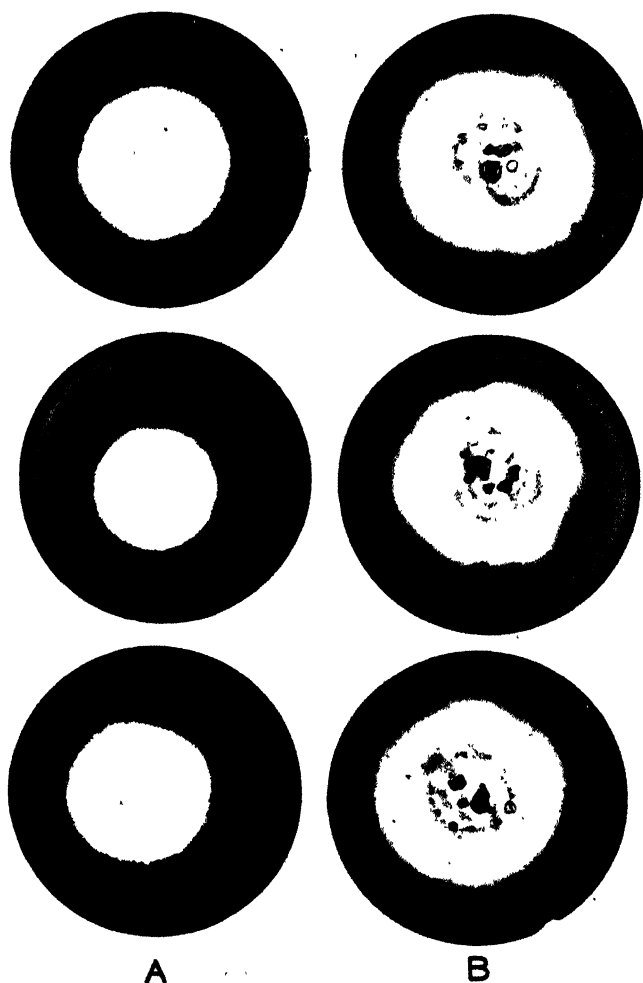


FIGURE 3.—Cultures, in triplicate, showing the uniformity of the gray (A) and the white (B) types of colonies derived from a monosporidial isolation of *Tilletia tritici* form T-3 (culture 263) and grown on potato agar containing 1 percent of dextrose, at 16° C.

added. Form L-1 of *T. levis* did not produce chromogenesis under any condition.

COMPARISON OF CULTURAL CHARACTERISTICS

The characteristics of 34 monosporidial cultures of seven physiologic forms of *Tilletia tritici* and *T. levis* were studied by growing them in triplicate at 16° C. in flasks of potato agar containing 1 percent of

dextrose. Figures 3 and 4 show that a fair degree of uniformity of cultural characteristics was obtained when care was exercised to control conditions and to select the inoculum. Figure 3 shows triplicate cultures of monosporial culture 263 of *T. tritici* form T-3. The colonies

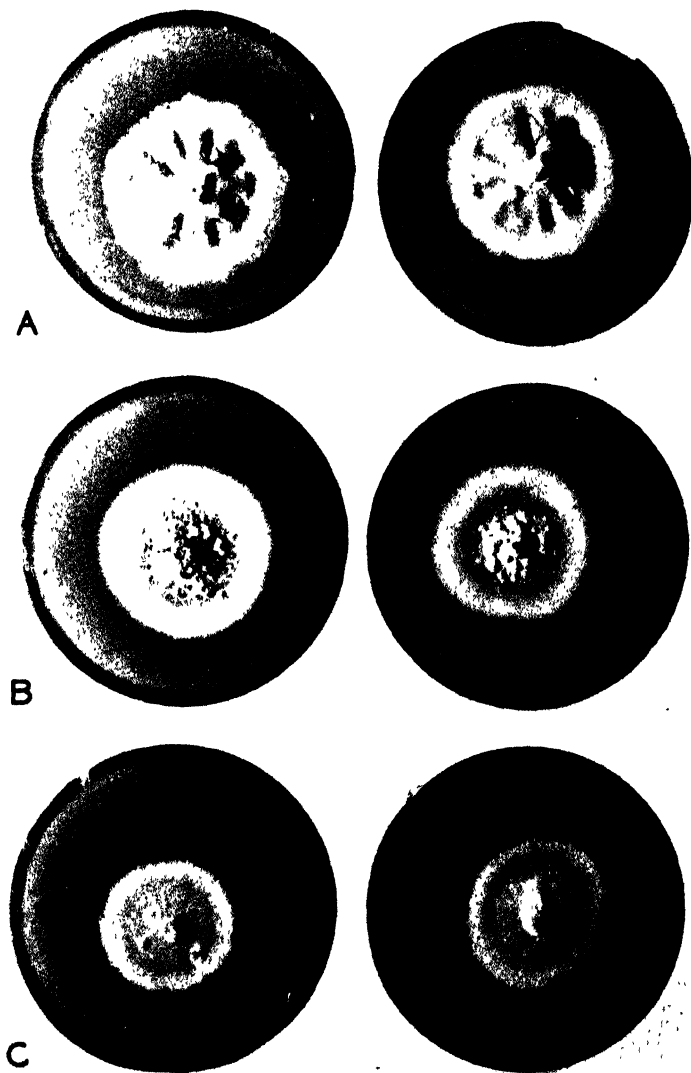


FIGURE 4 -- Duplicate cultures (side by side) of three monosporial isolations, (A) no. 225, (B) no. 284, and (C) no. 287, of *Tilletia teris* form L-2-c, grown on potato agar containing 1 percent of dextrose, at 16° C. The uniformity of cultures of the same monosporial isolation and the variability of cultures of monosporial isolations from the same pathogenic form are shown

produced from the gray mycelial inoculum are shown at the left (A). The mycelial inoculum for those at right (B) was obtained from raised white growths similar to those seen in the center of the gray colonies at left (A); that is, a culture derived from a single primary sporidium of

T. tritici produced colonies possessing distinctly different cultural characteristics. In figure 4 are shown in duplicate (side by side) cultures of three monosporidial isolations of *T. levis* form L-2-c derived from the same wheat head. Care was taken to secure the gray type of inoculum in each instance, and the uniformity of the inoculum was shown by the similarity of the two duplicate cultures in each case. The differences in the tendency of the individual cultures to produce the white and gray types of mycelium were marked. Culture 287 consisted almost entirely of the gray type, whereas culture 225 had changed to the white type, and culture 284 was composed of about equal parts of both gray and white. Although these cultures were obtained from the same physiologic form there was but little resemblance between them. As a matter of fact, culture 287 of form L-2-c of *T. levis* resembled the gray mycelial colony of culture 263 of form T-3 of *T. tritici* more closely than it did the other cultures of the physiologic form to which it belonged.

A study was made of the 12 monosporidial cultures of *Tilletia tritici* form T-3, whose paired pathogenicity had been determined (4). Six of these cultures belonged to sex group B (fig. 5) but showed little uniformity in cultural characteristics. Cultures 263 and 240 resembled one another somewhat, as did cultures 303, 304, and 306, but culture 243 was distinctly different from any of the others. Figure 6 shows the cultures of sex groups A, C, and D of form T-3. Cultures 213 and 209 of sex group A were fairly similar and resembled cultures 240 and 263 of sex group B, whereas cultures 302 and 305 of sex group D resembled one another and also resembled cultures 303, 304, and 306 of sex group B. Culture 235 of sex group C resembled somewhat cultures 302, 303, 304, 305, and 306 of sex groups A and B. Culture 262, which either belonged to another sex group or was sterile, differed culturally from all of the other cultures.

The monosporidial cultures of *Tilletia tritici* forms T-1 and T-2 and *T. levis* forms L-1, L-2-a, and L-2-b were as variable as those of *T. tritici* form T-3 and *T. levis* form L-2-c, with the single exception that cultures of *T. levis* form L-1 were not chromogenic. A preliminary study was made of the cultural characteristics of chlamydospore cultures of a number of collections in conjunction with field pathogenicity tests. No cultural characteristics were found that would aid in the identification of pathogenic forms, except that cultures of *T. levis* that were not chromogenic invariably belonged to the same pathogenic group as did *T. levis* form L-1. However, cultures of some collections that fell in the *T. levis* L-1 pathogenic group were chromogenic.

DISCUSSION

The problem of physiologic specialization of *Tilletia tritici* and *T. levis* is complicated by the limited knowledge of the genetics of these fungi. Rodenhiser (12) has shown the existence of numerous physiologic forms of a number of other cereal smuts by differences in cultural characteristics. However, since the mycelial stage of the bunt fungi is haploid (4) and the pathogenic and propagative phase is diploid (10), it is doubtful whether cultural characteristics should be considered the basis for the determination of physiologic forms.

The present studies have shown that different monosporidial cultures (haploid) derived from a single bunted head might be distinctly

different, despite the fact that the inoculum had been subjected to the purifying effects of varietal screening for several generations. As a

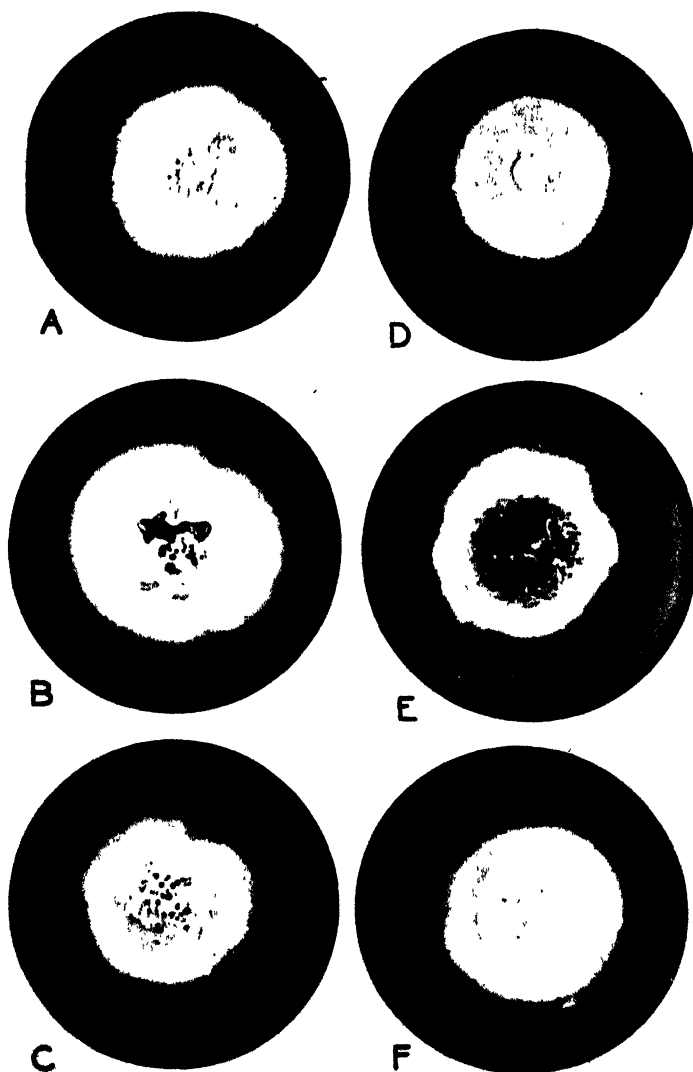


FIGURE 5.—Six monospore cultures, (A) no. 306, (B) no. 304, (C) no. 303, (D) no. 263, (E) no. 243, and (F) no. 240, of *Tilletia tritici* form T-3, sex group B, grown on potato agar containing 1 percent of dextrose, at 16° C, showing the lack of uniformity in cultural characteristics.

matter of fact, each one of the 12 cultures of a single pathogenic form might have been regarded as a distinct physiologic form on the basis of differences in cultural characteristics. In some cases a closer resem-

blance was found between the cultural characteristics of monosporidial cultures from the two species of *Tilletia* on wheat than between cultures derived from a single pathogenic form of either species. The

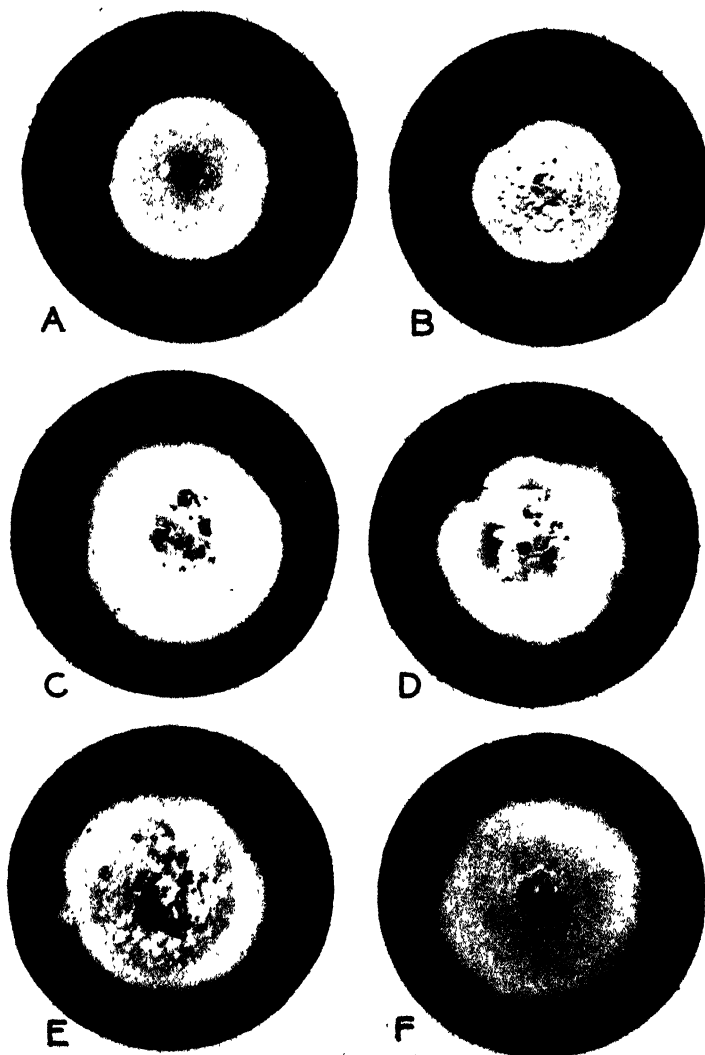


FIGURE 6—Six monosporidial cultures, (A) no. 209, (B) no. 213, (C) no. 302, (D) no. 305, (E) no. 235, and (F) no. 262, of *Tilletia tritici* form T-3 grown on potato agar containing 1 percent of dextrose, at 16°C., showing the different cultural characteristics of monosporidial isolations. Cultures 209 and 213 belonged to sex group A, nos. 302 and 305 to sex group D, no. 235 to sex group C, and no. 262 either was sterile or belonged to another group.

existence of great differences between the cultural characteristics of monosporidial cultures when derived from a single pathogenic form confirms the opinion that hybridization is the rule in the bunt fungi

and that a study of cultural characteristics with a view to determining pathogenicity is of little value, at least until pathogenic forms are based on something more homogeneous than field collections.

Up to the present time field collections of chlamydospores have been the basis for determining the physiologic forms of bunt. Usually if a series of more or less resistant differential varieties inoculated with a certain collection has produced percentages of infection sufficiently different from those produced by inoculation with other collections, the collection tested has been termed a new form. The only attempt at purifying the forms in a collection has been through a limited use of the "varietal screening" method. By this method the inoculum is increased on a resistant variety, and any included forms that are unable to attack this resistant variety are screened out.

The possibilities of the "varietal screening" method of form purification have just been touched upon, yet some significant results have been obtained. Bressman (2) and Weston (18) have shown that in some cases the original collection produced only a small percentage of bunt in Ridit, but when this bunt was again returned to this variety a strain was obtained which was capable of bunting Ridit severely. The value of varietal screening was further illustrated with Bressman's collection no. 28. Although he found a slight mixture of species in the collection, he reports it (2) as a form of *Tilletia levis* capable of infecting Oro severely and Albit and Hussar moderately. When this collection was increased on Oro, all the *T. tritici* was screened out, and the *T. levis* caused severe infection of Oro but was incapable of attacking Albit or Hussar. On the other hand, Oro was resistant to the *T. tritici* inoculum produced on Hybrid 128, but Albit and Hussar were severely bunted. Thus two strains of bunt having distinctly different pathogenic properties were separated from a single collection by the use of two varieties of wheat. It is possible that other pathogenically distinct strains might have been separated if additional varieties had been used as "screens." The purifying effect of increasing the inoculum on a resistant variety was illustrated by *T. tritici* form T-2, which had been increased on Hussar for a number of years. When inoculum of this form, obtained from Hybrid 128, Ridit, Oro, Hohenheimer, Albit, and Hussar, was again applied to these varieties there were no significant differences in pathogenicity. The propagation of this collection on its differential variety had purified it to such an extent that its pathogenicity was not altered by the screening effect of any of the resistant varieties on which it was subsequently propagated.

In the present study both *Tilletia levis* and *T. tritici* were found in 60 percent of the fields from which bunt collections were made throughout the wheat-growing areas of the Pacific Northwest. When it is considered that these collections consisted of from 50 to 100 heads taken from a very limited area of each field, it seems probable that both species of bunt may have been present in even a greater percentage of the fields. Besides the two species, apparently there are an indefinite number of physiologic forms in the Pacific Northwest area. The separation of the forms by varietal screening appears promising, but it will require a number of years to determine the value as well as the limitations of this method.

The problem of determining the number of physiologic forms in a collection is complicated not only by the possibility of physical

mixtures of forms, but also by the possibility of hybridization. It has been shown (4) that hybridization occurs between the two species *Tilletia levis* and *T. tritici*, as well as between the various forms within each species. The importance of hybridization under natural conditions is not known, but observational evidence (9) seems to indicate that it does occur.

The bunt survey of 1929 and 1930 showed that although *Tilletia tritici* continued to be the predominant species in the Pacific Northwest, *T. levis* was widely distributed and was present in all the major wheat-growing areas of that region. Pathogenicity tests of the collections showed that the less virulent strains of both species were most abundant. As a general rule, the less virulent form T-1 of *T. tritici* was found on bunt-susceptible varieties. When *T. levis* was obtained from susceptible varieties, it likewise usually proved to be the less virulent form L-1. As was to be expected, collections made from varieties resistant to the old forms usually contained the so-called "new," more virulent forms. The new forms were especially abundant in districts where the resistant varieties Albit and Redit were grown. They were also prevalent on susceptible as well as on resistant varieties on the Camas Prairie of Idaho and in the Grande Ronde Valley of Oregon, two districts considerably separated from the main wheat-growing area. In a few instances the new virulent forms were obtained from susceptible varieties in widely scattered localities in the main wheat-growing region. This indicated that the new forms had become established and would probably become prevalent over the entire region in a few years, unless methods of combating their spread were applied.

A number of means are available for retarding the dissemination of these new physiologic forms, but their application requires the intelligent cooperation of all wheat growers in the regions concerned. In the first place, locally grown seed should be used wherever possible; and, secondly, all seed, whether locally grown or imported, should be efficiently treated, as has been recommended by Heald and Gaines (6) and Holton (8). This applies to seed of resistant varieties as well as to that of susceptible varieties, since the destruction of seed-borne spores would obviate to a considerable extent the screening effect of resistant varieties whereby forms of bunt capable of infecting them are propagated. A third possibility is the intelligent use of a rotation of resistant wheat varieties. For example, at the present time no form capable of attacking both Turkey selection (C.I. 7366) and Albit is known. If Albit should become bunted in one locality, a concerted change, for 1 or 2 years, to Turkey selection (C.I. 7366) or to a variety possessing similar resistance should eliminate those forms capable of attacking Albit.

It has been shown that forms of bunt capable of infecting Albit and Redit are rather prevalent in fields of these varieties. Except in a few instances, these forms are not sufficiently abundant to cause serious economic losses at present (1931), and consequently seed treatment often is not practiced. Unless an adequate seed-treatment program is adopted it seems probable that it will be only a few years until the more virulent forms will become abundant enough to cause serious losses to these resistant varieties and perhaps to others that may be developed in the future.

SUMMARY

Bunt collections were made from 182 fields in the principal wheat-producing areas of Oregon, Washington, and northern Idaho.

Tilletia tritici was found in every collection, whereas *T. levis* was present in only 60 percent of the collections. Of the two species, *T. levis* was the more abundant west of the Cascade Mountains and was present in all the wheat-growing regions, although *T. tritici* was the prevailing species east of the Cascades.

Pathogenicity tests of the collections indicated that at least 7 forms of *Tilletia tritici* and 6 forms of *T. levis* were present. In some collections there appeared to be an intergrading of one form with another, indicating form mixtures or hybridization, whereas other collections had sharply differentiated pathogenic properties.

Eighty-four percent of the collections contained only the less virulent forms of *Tilletia levis* or *T. tritici*, forms unable to infect resistant varieties such as Albit, Redit, Hussar, and White Odessa.

Although most of the new virulent forms were obtained chiefly from fields of Albit and Redit, they were also found in a few collections from susceptible varieties in widely scattered localities.

The presence of the new virulent forms of both *Tilletia levis* and *T. tritici* on susceptible as well as on resistant varieties makes the adoption of cultural practices imperative in order to impede the spread of these more virulent forms.

Pathogenically distinct forms were readily separated by the screening effect of resistant varieties. However, varietal screening had no effect on the pathogenicity of a collection propagated on a resistant variety, such as Hussar, for several years.

Morphological variations such as size, color, and character of the reticulations of the spore wall, as well as hardness of the bunt ball, were found to be heritable, but no definite correlation was established between these characteristics and pathogenicity.

Variation of the temperature during the period of infection did not cause significant differences in the percentage of bunt produced by five pathogenically distinct physiologic forms.

The characteristics of monosporidial cultures of *Tilletia levis* and *T. tritici* were exceedingly variable. In some instances monosporidial cultures of the two species resembled one another more closely than did those from the same pathogenic form.

No correlation was obtained between cultural characteristics and pathogenicity, except that nonchromogenic cultures of *Tilletia levis* invariably belonged to the less virulent pathogenic form that did not attack Albit, Hussar, or White Odessa.

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A MORPHOLOGICAL STUDY OF FLOWER AND SEED DEVELOPMENT IN CABBAGE¹

By ROSS C. THOMPSON

Associate horticulturist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The solution of many of the problems associated with cabbage-seed growing and commercial cabbage production lies in the field of plant breeding. Few of the vegetable crop plants are so variable as the cabbage family—the Cruciferae. Most of the common varieties of cabbage (*Brassica oleracea capitata* L.) are lacking in uniformity. There is an urgent demand on the part of both seedsmen and commercial cabbage growers for a standardization of varieties, so that a variety will represent a definite type, rather than a mixture of various types as is now so often the case.

It has been demonstrated that at least some of the cabbage diseases can be controlled by the use of strains selected for their resistance to these diseases. Most of the common cabbage varieties can no doubt be greatly improved by scientific methods of breeding and selection.

Any attempt to solve these problems through breeding methods requires a knowledge of the morphology of the cabbage flower. The method of pollination, the time required for fertilization, and a general knowledge of the flower structure and its development are important in a breeding program. It was for the purpose of answering some of the questions relating to the cabbage flower and its development that this study was undertaken.

MATERIAL AND METHODS

The first material was collected during the winter of 1929 and the spring of 1930. The plants were grown from seed of the Early Jersey Wakefield variety planted September 4, 1929. All material used in these studies was grown at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D.C. When the plants were about 3 inches high they were transplanted to the field, where they were overwintered. Material for study was collected at intervals of 2 weeks from November 15 to April 25.

A second lot of plants of the same variety was started from seed on August 25, 1930. The plants were set in the field and overwintered as in 1929. Material was collected at intervals of 2 weeks from the middle of November to the latter part of May.

Material was also collected during the fall and winter of 1930 and 1931 from mature plants which were set in the field in the summer of 1930.

The tissues were placed immediately in a killing fluid. Several such agents, including Bouin's, Carnoy's, medium chromoacetic acid, and formalin acetic alcohol were used in the early part of the work. It was later found necessary to use a killing agent in which the tissues could be kept for some time without injury, as it was not convenient to carry the materials through the dehydrating, clearing, and embedding processes immediately after they were collected. The formalin acetic alcohol solution, made up from 50 cc of 95 percent

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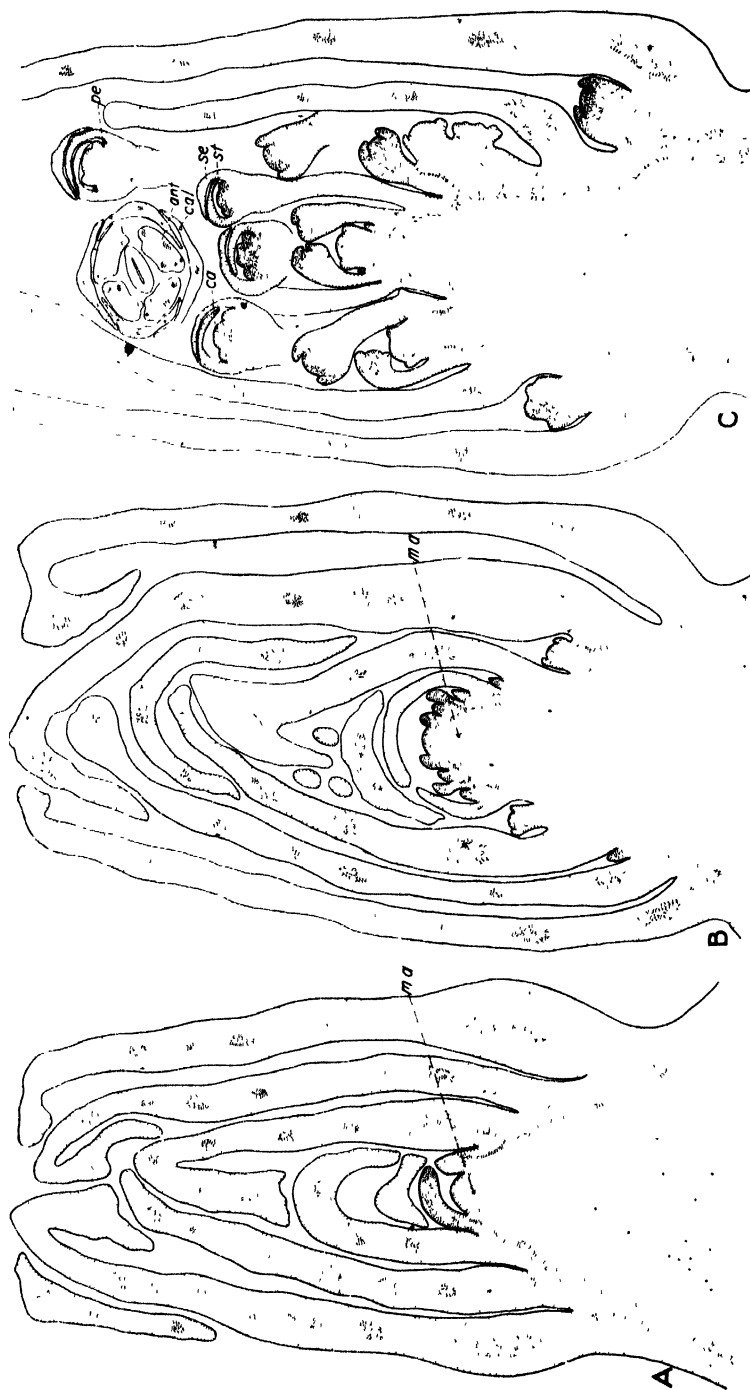


FIGURE 1 — Main axis of cabbage stem. A, Before differentiation of flower parts. B, in early stage of flower differentiation. C, in late stage of flower development. $\times 16$
(For explanation of abbreviations see opposite page.)

alcohol, 5 cc glacial acetic acid, 5 cc commercial formalin, and enough distilled water to bring the total volume up to 100 cc, was found to be satisfactory for this purpose. Flemming's weaker osmic acid fixing agent was employed for anthers in which division of the pollen mother cells was to be studied.

The waxy epidermis of *Brassica* is very resistant to penetration of the killing agent. Air bubbles under the bracts of the flower also interfered with the killing process. The difficulty in securing rapid penetration and killing was overcome by subjecting the material to a condition of partial vacuum immediately after placing it in the solution.

After dehydrating in a series of alcohols of varying concentration, including absolute alcohol, the materials were cleared in xylol and embedded in paraffin having a melting point of about 55° C.

Delafield's haematoxylin was found to be the most satisfactory stain for most of the structures studied. Heidenhain's iron-alum haematoxylin was used for embryo sacs and for the various stages of pollen development.

DIFFERENTIATION OF FLOWER PRIMORDIA

The time of differentiation of flower primordia in cabbage has been found to depend very largely on temperature relations. Miller² has shown that the cabbage plant is capable of continuous vegetative growth without seed formation, at least for several years, if the plant is never subjected to low temperature. Boswell³ found that the differentiation of flower primordia in young cabbage plants is closely correlated with the size of the plant at the time it is exposed to low temperature. Low temperature failed to induce flower development unless the plant had reached a certain size as indicated by a stem diameter of about 6 mm.

Many sections were prepared from material collected from the plants started from seed on September 4, 1929, and August 25, 1930. The first indication of differentiation of flower primordia in the material started September 4, 1929, was found in terminal buds killed March 3. In the material started from seed on August 25, 1930, the first differentiation was found in material killed February 23. This confirms the findings of Boswell³ that differentiation in immature cabbage plants overwintered in the open takes place in early spring.

² MILLER, J. C. A STUDY OF SOME FACTORS AFFECTING SEED-STOCK DEVELOPMENT IN CABBAGE. N. Y. (Cornell) Agr. Expt. Sta. Bul. 488, 46 p., illus. 1929

³ BOSWELL, V. R. STUDIES OF PREMATURE FLOWER FORMATION IN WINTERED-OVER CABBAGE. Md. Agr. Expt. Sta. Bul. 313, p. 69-145, illus. 1929

EXPLANATION OF ABBREVIATIONS IN ILLUSTRATIONS

<i>a c</i> , Archeporsporial cell	<i>hyp</i> , Hypocotyl	<i>p m c</i> , Pollen mother cells
<i>an</i> , Antipodals	<i>hypo</i> , Hypophysis	<i>p e</i> , Points of exit
<i>ant</i> , Anther	<i>i i</i> , Inner integument	<i>pol</i> , Pollen
<i>ca</i> , Carpels	<i>m</i> , Megaspore	<i>pol tu</i> , Pollen tube
<i>cal</i> , Calyx	<i>m a</i> , Main axis	<i>r c</i> , Rootcap
<i>col</i> , Cotyledons	<i>m l</i> , Middle layer	<i>sc</i> , Sepals
<i>cyt</i> , Cytoplasm	<i>m m c</i> , Megaspore mother cell	<i>sp</i> , Spermis
<i>der</i> , Dermatogen	<i>mi</i> , Micropyle	<i>st</i> , Stamens
<i>d m</i> , Disintegrating megaspores	<i>nu</i> , Nucellus	<i>st l</i> , Stigmatic lobe
<i>egg nu</i> , Egg nucleus	<i>o</i> , Ovary	<i>sty</i> , Style
<i>emb</i> , Embryo	<i>o i</i> , Outer integument	<i>sup la</i> , Supporting layer
<i>emb c</i> , Embryo cell	<i>ov</i> , Ovule	<i>sus</i> , Suspensor
<i>emb f</i> , Embryo filament	<i>o w</i> , Ovary wall	<i>syn</i> , Synergids
<i>en</i> , Endothecium	<i>p nu</i> , Polar nuclei	<i>ta</i> , Tapetum
<i>end</i> , Endosperm	<i>pe</i> , Petals	<i>te</i> , Tetradis
<i>ep</i> , Epidermis	<i>peri</i> , Periblem	<i>vac</i> , Vacuole
<i>epi</i> , Epicotyl	<i>pig la</i> , Pigmented layer	<i>v n</i> , Vegetative nucleus
<i>e s</i> , Embryo sac	<i>pl</i> , Placenta	
<i>fu</i> , Funiculus	<i>pl</i> , Plerome	

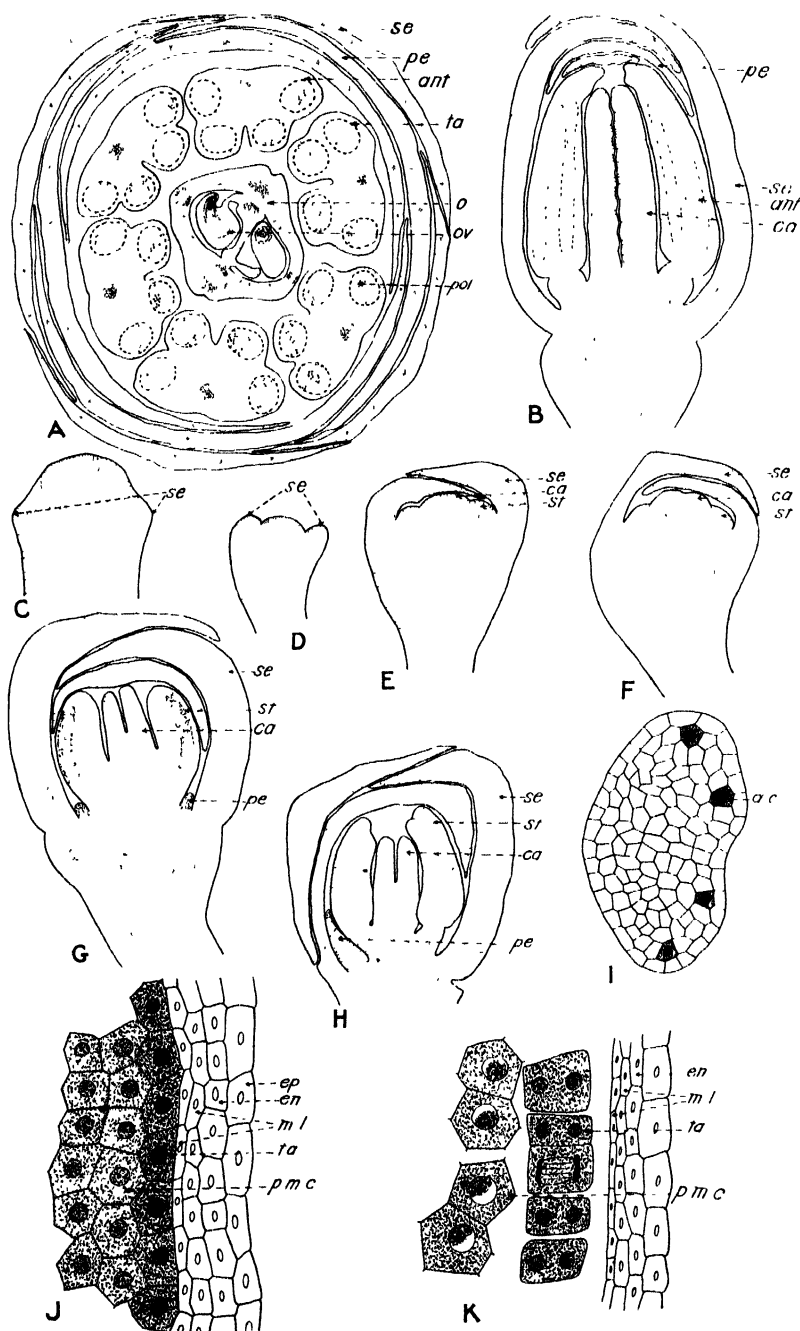


FIGURE 2.

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The first indication of flower development in material collected from mature plants during the fall of 1930 was found in sections prepared from terminal buds killed October 28.

It is evident that the time of differentiation of flower primordia in cabbage varies with the age of the plant and the seasonal conditions. In the latitude of Washington, D.C., flower primordia in mature cabbage plants differentiate in the fall; immature plants overwintered in the open produce flower primordia in early spring.

The first indication of flower differentiation is suggested by the elongation of the entire terminal region and by the change in contour of the surface of the growing apex. Three stages in the development of the apex of the main axis are shown in figure 1. Figure 2, A and B, shows the general structure of the cabbage flower. The growing apex, at first flattened (fig. 1, A), becomes conical and soon gives rise to protuberances near the margin (fig. 2, C and D). The first whorl of swellings gives rise to the four sepals. Unless the temperature is too low for growth, the differentiation of the various organs progresses rapidly. The sepals are followed by a second whorl of swellings on the marginal surface of the apex (fig. 2, E, *st*), which produces the six stamens. The carpels are next in order of appearance and arise from the growing apex at its junction with the whorl of stamens (fig. 2, F, *ca*). The petals are the last cycle of flower organs to appear. They are first evidenced by swellings at the base of the stamens between the stamens and the sepals (fig. 2, G and H, *pe*). The succession of development of the various cycles agrees with that of *Bursa (Capsella) bursa-pastoris* (L.) Britton, in which the order deviates from the acropetalic succession characteristic of many cyclic flowers.

DEVELOPMENT OF MICROSPORANGIA

The anthers at first consist of a homogeneous mass of meristematic cells.

The anthers produce four microsporangia each. The archesporial cells which give rise to the pollen mother cells in each locule of the anther are cut off from a row of hypodermal cells under each of the four lobes. The archesporial cells are distinguishable when the anthers have developed to the stage indicated in figure 2, I. The archesporial cells divide by periclinal walls, forming an inner layer of cells that give rise to the pollen mother cells, and an outer layer that divides to produce the endothecium, one or more middle layers, and the tapetum (fig. 2, J and K). The pollen mother cells and the tapetum are readily distinguished by their difference in reaction to stains. The tapetum consists of a single layer of cells at first uninucleate (fig. 2, J, and fig. 3, B), but they soon become binucleate

EXPLANATORY LEGEND FOR FIGURE 2

- A, Transverse section through cabbage flower. $\times 40$. B, Longitudinal section of a flower in late stage of development. $\times 20$. C, Growing apex; sepals differentiating. $\times 40$. D, A more advanced stage of sepal development. $\times 33$. E, Stamen differentiation. $\times 33$. F, Differentiation of carpels. $\times 33$. G, Carpels and petals differentiated. $\times 36$. H, A more advanced stage of petal development. $\times 20$. I, Transverse section through young anther showing differentiation of sporogenous cells. $\times 150$. J, Longitudinal section through an anther in which the pollen mother cells, tapetum, middle layers, and endothecium have differentiated. $\times 240$. K, Longitudinal section of an anther. Tapetum binucleate and pollen mother cells beginning to separate. $\times 240$.

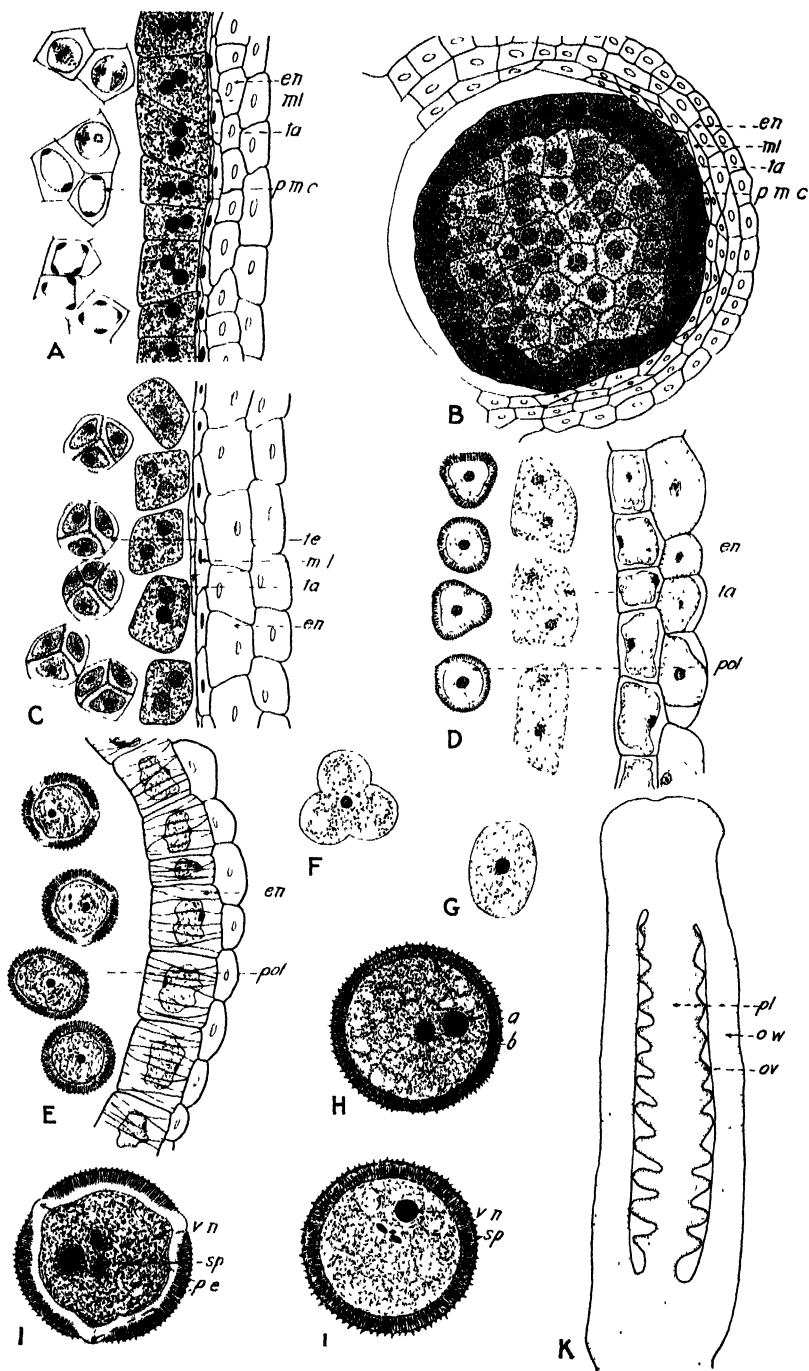


FIGURE 3

For explanatory legend see opposite page.

(fig. 2, K). The sporogenous cells continue to divide in all planes until in transverse section the plate of pollen mother cells is 6 to 8 cells in diameter (fig. 3, B). In the homotypic division of the pollen mother cells (fig. 3, A), the spindles may extend in either opposite or parallel planes. Absorption of the middle layer or layers often begins by the time the pollen mother cells have reached the tetrad stage (fig. 3, C.) The middle layer may remain intact until the pollen mother cells are well developed, having thick, spiny exine (fig. 3, D). The variation in the absorption of the middle layer indicates the great variation in degree of many of the stages of development of the various structures. The endothecium exhibits a similar variation in time of enlargement and thickening of the cell walls in relation to the development of other layers and to pollen development. At maturity the endothecium cells are very much enlarged and have numerous radial bands of thickening in their walls (fig. 3, E).

The pollen mother cells in the tetrad stage are shown in figure 3, C. After their separation from the tetrad they are very thin walled (fig. 3, F and G); in the later stages a thick-walled spiny exine develops (fig. 3, H and J). There are three points of exit or thin areas in the exine through one of which the pollen tube emerges when the pollen grain germinates (fig. 3, I). At the time the young pollen grains are freed from the surrounding membrane in which they are held in the tetrad stage they are uninucleate. In figure 3, H, the nucleus of the pollen grain has divided to form the vegetative nucleus (*a*) and the generative nucleus (*b*). Before maturity the generative nucleus divides to give rise to the two sperms (fig. 3, I and J).

The microsporangia develop considerably before the megasporangia. Pollen cells were found beyond the tetrad stage and in some cases well developed by the time of the first division of the archesporial cells of the ovules. At the time of the first division of the megaspore mother cell the exine of the pollen grains has become thickened and the nucleus has divided to produce the vegetative and generative nuclei. The stages of development of the microsporangia and the megasporangia do not always advance at the same rate. Considerable variation in stages of development was found in material of about the same age but killed on different days.

Figure 4, E, shows a longitudinal section through an immature stigma. The stigmatic lobes are covered by long, irregular-shaped cells that produce a very rough surface. The indentations between the protruding stigmatic cells provide a lodging place for the pollen grains until they germinate and the pollen tubes have penetrated the style.

EXPLANATORY LEGEND FOR FIGURE 3

A, Longitudinal section through an anther. Pollen mother cells in homotypic division. Inner middle layer disintegrating. $\times 240$. B, Transverse section of a single locule of an anther in same stage as figure 2, J. The various tissues have all differentiated. $\times 200$. C, Longitudinal section through an anther. Pollen mother cells in tetrad stage. $\times 240$. D, Longitudinal section of an anther. Pollen cells nearing maturity; middle layers have disintegrated; tapetum breaking down; cells of the endothecium beginning to enlarge. $\times 240$. E, Longitudinal section of an anther. Pollen cells mature; tapetum entirely disintegrated and absorbed; endothecium cells much enlarged and heavily ribbed. $\times 240$. F, Median section through a pollen grain after liberation from the tetrad, showing thin wall and three constrictions where the points of exit develop in the mature grain. $\times 390$. G, Section through a pollen grain in the same stage as F but passing through a plane perpendicular to F. $\times 390$. H, Median section through a pollen grain, showing vegetative nucleus (*a*) and sperm nucleus (*b*). $\times 390$. I, Median section of a mature pollen grain through the three points of exit, showing the vegetative nucleus and the two sperms. $\times 390$. J, Median section of a mature pollen grain in which none of the points of exit have been bisected. $\times 390$. K, Longitudinal section through an ovary, showing the ovules developing in each of the two carpels. $\times 33$.

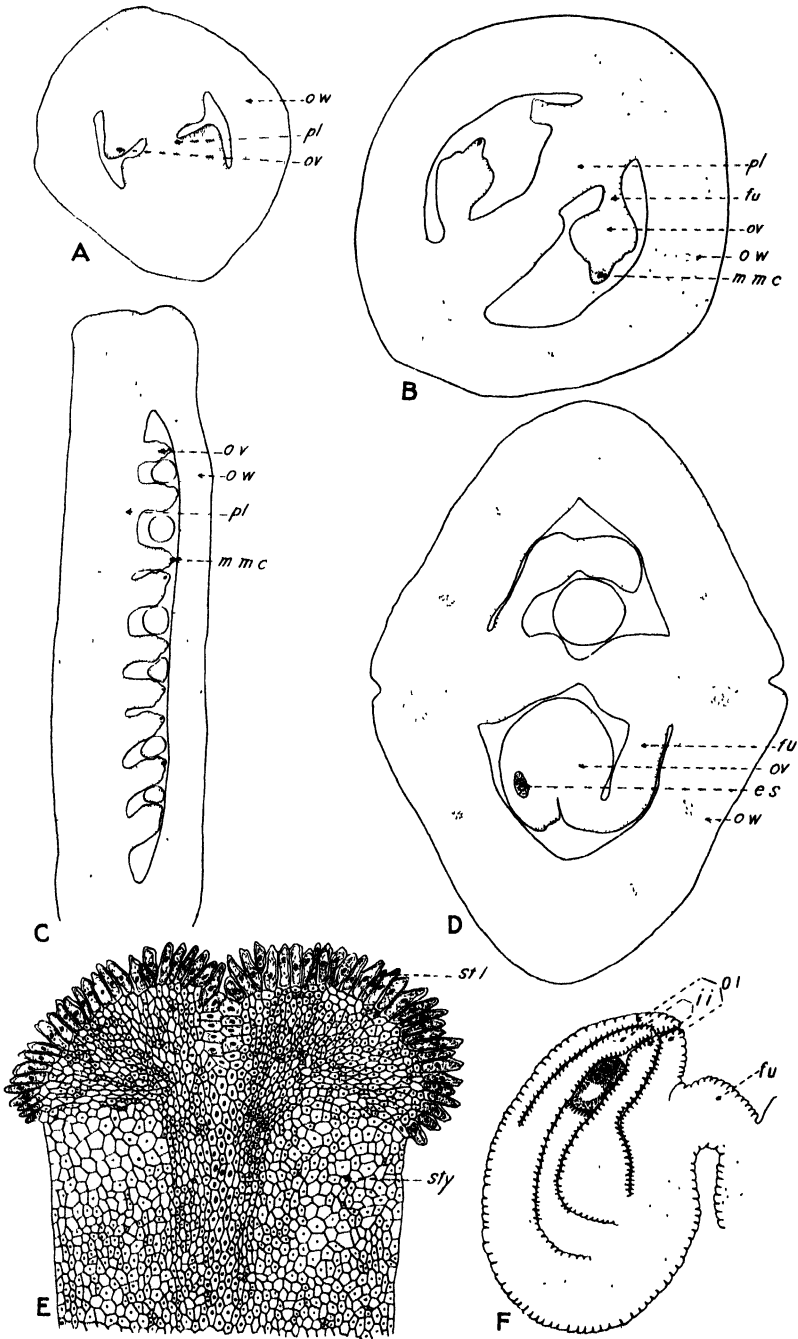


FIGURE 4.

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THE MEGASPORANGIUM AND THE EMBRYO SAC

The ovules develop from protuberances that arise from the placenta near its junction with the ovary wall. Two rows of swellings develop in each of the two carpels (fig. 3, K, and fig. 4, A). By cell division the protuberances become increasingly prominent and constitute the nucellus of the nascent ovule. When the enlarging nucellus has reached the stage indicated in figure 4, B and C, the hypodermal archesporial cell is readily distinguishable by its greater size and larger dark-staining nucleus (fig. 5, A). In some cases the archesporium is 2-celled (fig. 5, B), but only one of these cells ever becomes a megaspore mother cell. As in other Cruciferae, *Bursa* (*Capsella*) in particular, as reported by Guignard,⁴ there is no parietal cell. Figure 4, D and F, shows the campylotropous structure of the ovule.

When the developing ovule has attained the stage shown in figure 5, A, the integument swellings are evident. In this case there are two, an inner and an outer integument. The two integuments are differentiated almost simultaneously, the inner one generally slightly before the outer. They develop very rapidly by cell enlargement and division and soon completely enclose the nucellus (fig. 5, C, D, and F). Figure 5, E, is a transverse section through an ovule in the same stage as figure 5, D. At this stage the nucellus and inner integument each consist of two layers of cells and the outer integument of three layers. By two successive divisions the megaspore mother cell produces a linear row of four megaspores (fig. 5, F), only one of which, usually the one toward the chalazal end, continues to function. The three micropylar megaspores soon disintegrate and are absorbed by the remaining functional megaspore (fig. 5, G), which develops rapidly and increases considerably in size before the first division of its nucleus. The nucleus is in a central position in the cell at the time of the first division (fig. 5, H). The resulting daughter nuclei separate, one migrating to the micropylar end and the other to the chalazal end of the sac (fig. 5, I). Figure 5, J, shows the four nuclei resulting from the first division of the two daughter nuclei. By two successive divisions the two nuclei divide to produce eight free nuclei (fig. 5, K). Two of the eight nuclei take a position near the center of the sac and become the polar nuclei (fig. 6, A); three migrate to the extreme chalazal end of the sac and become antipodals (fig. 6, B); the three remaining take a position near the micropylar end of the sac; the two nearest the micropyle become synergids, and the innermost one becomes the egg. The complete embryo sac at first develops most rapidly at its micropylar end (fig. 6, B). At this stage the embryo sac is pear-shaped, being much enlarged at its micropylar end and tapering toward the chalazal end. From this point on the greatest enlargement occurs at the chalazal end, while the micropylar end becomes a narrow neck (fig. 6, H and I).

⁴ GUIGNARD, L. RECHERCHES SUR LE SAC EMBRYONNAIRE DES PHANÉROGAMES ANGIOSPERMES. *Ann. Sci. Nat., Bot.* (6) 13 [136]-199, illus.

EXPLANATORY LEGEND FOR FIGURE 4

- A, Transverse section through an ovary at about the same stage of development as in figure 3, K. $\times 70$. B, Transverse section through an ovary, showing the differentiation of the archesporial cell. $\times 90$. C, Longitudinal section of an ovary in the same stage as in B. $\times 33$. D, Transverse section of an ovary, showing the campylotropous ovule. $\times 33$. E, Longitudinal section through a stigma, showing the structure of the stigmatic surface. $\times 90$. F, Section through an ovule and its funiculus, showing the campylotropous structure and the two integuments. $\times 70$.

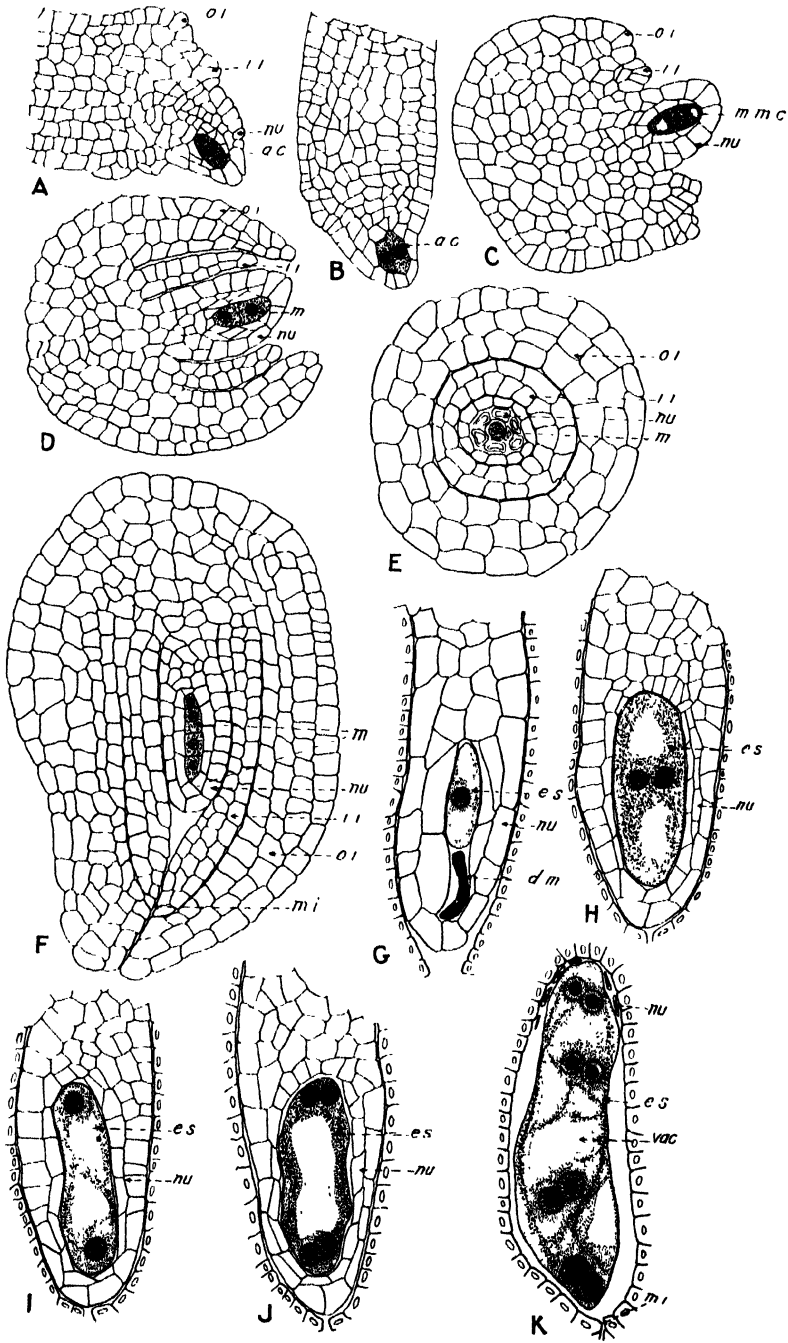


FIGURE 5.

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The sac enlarges rapidly and constitutes a very large portion of the developing ovule. As the sac enlarges, the cytoplasm becomes increasingly vacuolated. A large central vacuole soon forms, and the cytoplasm is forced to the outer wall (fig. 6, G, H, and I).

The antipodals are short-lived, soon disintegrating. They may become multinucleate before disintegration (fig. 6, D). The antipodals in this case apparently take no part in the activities of the developing embryo sac. Like the antipodals, the synergids are ephemeral and are soon absorbed by the developing sac. Many cases were noted in which the embryo sac and the nucellus had not developed and fertilization had evidently failed to take place. However, the integuments had continued to enlarge by cell division, and the ovules in some cases were as large as the fertilized ovules, but in the later stages these unfertilized ovules shriveled and died. The reason for the failure of certain egg cells to be fertilized was not determined.

As shown by figure 6, C and D, the pollen tube may enter from either the micropylar or the chalazal end of the sac. In most cases the tube was found to enter from the micropylar end.

The structure of the cabbage flower is such that under field conditions the ovules may be either selfed or cross-fertilized. The flowers are visited by various insect species. When the seed-producing plants are grown in the open and unprotected from insects, the percentage of cross-fertilization is likely to be quite high. Where self-pollinated seed is desired it is necessary that the plants be protected from insects. The statement is often made that a single cabbage plant rarely produces seed, or is self-sterile. Some strains are doubtless largely self-sterile, but this is not true of all. In this study no difficulty was experienced in obtaining fertile ovules that matured normal seed when the flowers were artificially selfed.

EMBRYO DEVELOPMENT

There appears to be some variation in the time elapsing between pollination and fertilization. Considerable variation was noted in stages of development of ovules. Within a single carpel ovules were found in which the endosperm had completely lined the wall of the embryo sac; in other ovules in the same carpel fertilization had not yet taken place. The first fertilized eggs were found in material killed 4 days after pollen had been applied to the stigmas, but in most cases fertilized eggs and pollen tubes within the sac were found in material killed 5 or 6 days after pollination. A wall is formed around the egg soon after fertilization. The synergids disintegrate about the time the egg is fertilized (fig. 6, E). The endosperm nucleus divides slightly in advance of the fertilized egg (fig. 6, E). The fertilized egg cell, by several successive divisions, produces a linear row of cells (fig. 6, F and H). The filament usually consists of 6 or 8 cells before the terminal or embryo cell divides (fig. 6, H, I, and J).

EXPLANATORY LEGEND FOR FIGURE 5

- A, Section through a young ovule, showing the archesporial cell and the developing integuments. $\times 180$. B, Section through an ovule, showing 2-celled archesporium. $\times 150$. C, Section through a young ovule just before the first division of the sporogenous cell. $\times 120$. D, Ovule showing development of integuments after the first division of the sporogenous cell. $\times 120$. E, Transverse section through an ovule in the same stage as D. $\times 120$. F, Ovule showing the four megaspore mother cells resulting from division of the archesporial cell shown in A. $\times 120$. G, Section through an ovule, showing disintegration of the three nonfunctioning megaspores. $\times 180$. H, Embryo sac after the first division of its nucleus. $\times 180$. I, Embryo sac; the two nuclei have moved to opposite poles. $\times 180$. J, Embryo sac in 4-nucleate stage. $\times 180$. K, Embryo sac with eight nuclei. $\times 180$.

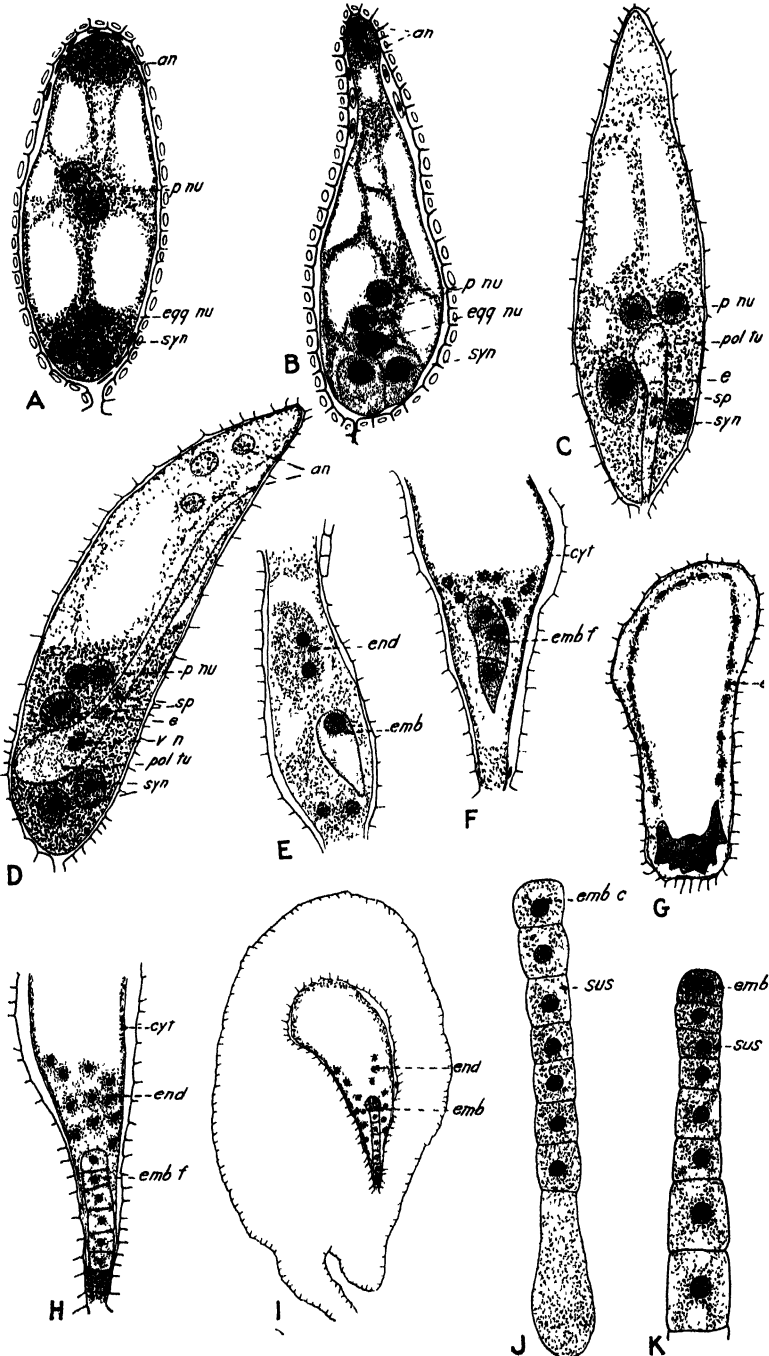


FIGURE 6.

For explanatory legend see opposite page.

In this discussion the terminal cell of the filament is considered as the "embryo cell," although, as in *Bursa*, the first cell of the suspensor contributes to the embryo tissues. Some of the cells of the suspensor filament undoubtedly continue to divide by transverse walls after the first division of the embryo cell, for never more than 8 cells were found in the filament at this stage; yet in later stages in the embryo development suspensors were found with 10 and 12 cells. The first division of the embryo cell is longitudinal (fig. 6, K). The two daughter cells by longitudinal and transverse walls divide to produce the 8-celled or octant stage (fig. 7, A). The first transverse walls separate the cotyledonary from the hypocotyledonary regions of the embryo. The cotyledons and the epicotyl develop from the four apical octants shown in figure 7, A, *a*. As in *Bursa*, a portion of the hypocotyl is derived from the hypophysis, which is cut off from the suspensor by a transverse division of the cell adjacent to the embryo (fig. 7, C). The hypocotyl then is derived from the portion *b* and a part of cell *c* in figure 7, A.

The dermatogen is differentiated early in the development of the embryo (fig. 7, B), except for that portion derived from the hypophysis, and is soon completed. The plerome and periblem differentiate when the embryo has reached the stage indicated in figure 7, C. The portion of the embryo *a* in figure 7, A, develops more rapidly than *b*, as indicated in figure 7, D and E. The suspensor cell adjacent to the embryo has divided by a transverse wall. The daughter cell next to the embryo contributes to the periblem and dermatogen of the rootcap; the other plays no part in the embryo development but remains as part of the suspensor.

The first division of the hypophysis is transverse. The daughter cell adjacent to the embryo contributes to the periblem of the root (*a*) in figure 7, D; the basal daughter cell (*b*) produces a plate of four cells by two longitudinal divisions. The longitudinal wall in cell *b* precedes the longitudinal division of cell *a* in figure 7, E. A transverse division of the plate of four cells in *b*, figure 7, F, results in a second plate of four cells *c* in figure 7, G. The four cells in *b*, figure 7, G, complete the dermatogen of the root tip. The dermatogen, which is the first tissue to be differentiated, is the last to be completed. The cells in (*c*) of figure 7, G, become the first layer of the rootcap shown in figure 7, H. By transverse divisions of this layer of cells other layers are added to complete the rootcap, as indicated in figure 7, I and J. The development of the embryo follows very closely that reported by Hanstein⁵ and Famintzin⁶ for *Capsella*.

⁵ HANSTEIN, J. ENTWICKLUNG DES KEIMES DER MONOKOTYLEN UND DIKOTYLEN. *1878* Botanische Abhandlungen aus dem Gebiet der Morphologie und Physiologie, Bd. 1, Heft 1, 112 p., illus. Bonn. 1870

⁶ FAMINTZIN, A. EMBRYOLOGISCHE STUDIEN. *Mem Acad Imp Sci St Petersburg*, 26, no 10. 1879

EXPLANATORY LEGEND FOR FIGURE 6

A, Embryo sac; the polar nuclei have moved to the chalazal end of the sac and the synergids toward the micropyle. $\times 180$. B, Embryo sac just before the disintegration of the polar nuclei. $\times 180$. C, Embryo sac in which the pollen tube has entered from the micropylar end. $\times 180$. D, Embryo sac in which the pollen tube has entered from the chalazal end. Antipodals multinucleate. $\times 180$. E, Embryo sac after fertilization. The endosperm nucleus has completed its first division; synergids disintegrating. $\times 200$. F, Embryo sac with embryo in 3-celled stage. From material killed 5 days after pollination. $\times 200$. G, Embryo sac showing mass of endosperm at chalazal end. $\times 40$. H, Embryo in the 8-celled filament stage. From material killed 8 days after fertilization. $\times 120$. I, Section through an ovule, showing embryo in the octant stage. Killed 10 days after pollination. $\times 33$. J, Embryo filament before division of the terminal or embryo cell. Killed 8 days after pollination. $\times 200$. K, First division of embryo cell. Killed 8 days after pollination. $\times 200$.

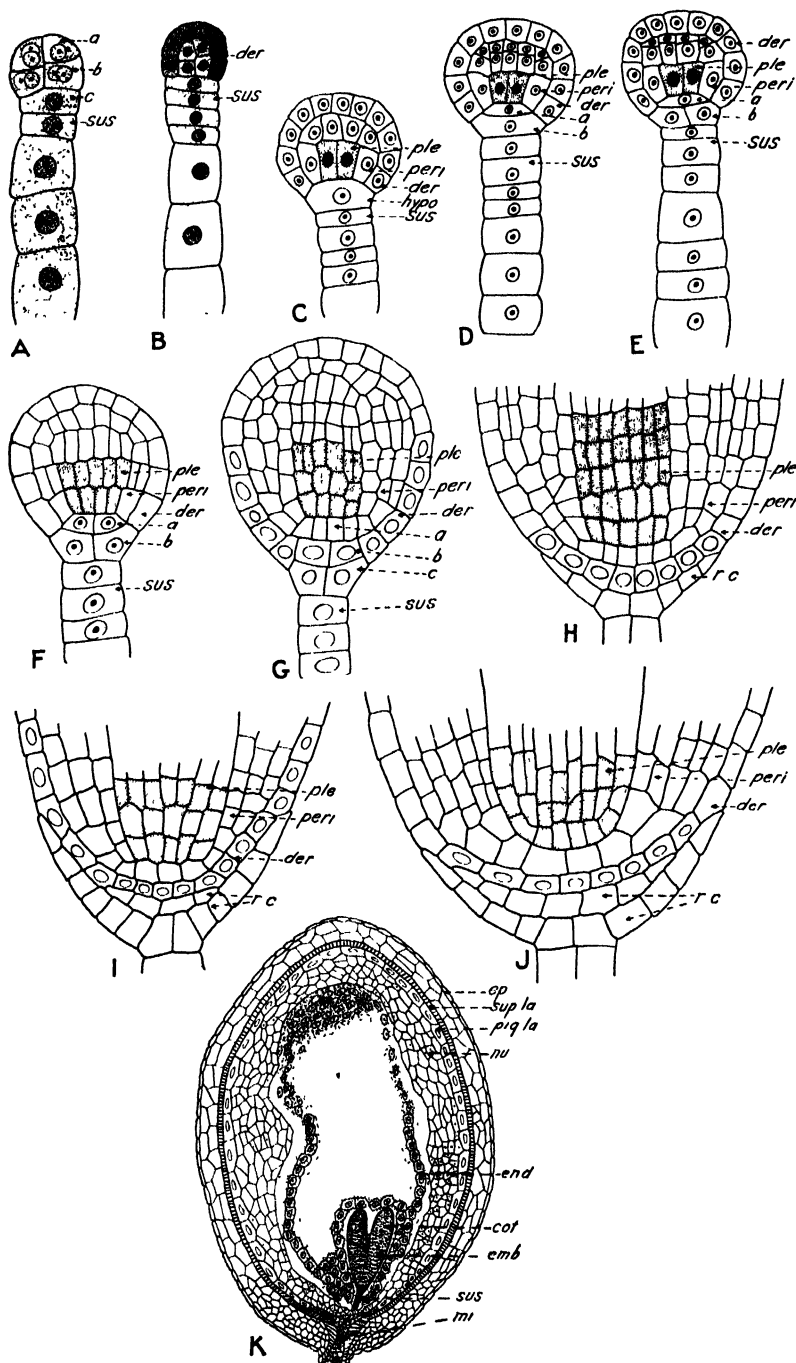


FIGURE 7.

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THE ENDOSPERM

The primary endosperm nucleus divides before the egg. Further divisions of the endosperm follow in rapid succession, hence it develops in advance of the embryo.

Many of the nuclei divide simultaneously, as in some sections nearly all of the nuclei present were in some phase of cell division. The nuclei are at first free without separating walls. As the embryo develops, the endosperm surrounding the embryo becomes separated by walls, while some of the nuclei at the chalazal end apparently never become separated. As the vacuolation of the sac increases and the cytoplasm is driven outward against the wall of the sac, the endosperm forms a single cell layer around the wall of the sac and is embedded in the limited cytoplasm. Generally, a considerable mass of endosperm develops at the extreme chalazal end of the sac (fig. 6, G), and to a less extent around the developing embryo (fig. 7, K, and fig. 8, A). No very large amount of endosperm tissue ever develops, and that which does develop is soon absorbed by the growing embryo; hence the seed of cabbage is without endosperm.

THE SEED

The embryo constitutes the largest portion of the mature ovule or seed (fig. 8, B and C). As the embryo develops and expands it fills the large vacuole of the embryo sac and absorbs the cytoplasmic contents of the sac as well as the endosperm. At maturity the endosperm has disappeared and most of the nucellar tissue has been absorbed. The stored nutrient materials for the young seedling at the time of germination are carried by the cotyledons and hypocotyl of the embryo. Surrounding the well-developed embryo are the seed coats derived from the two integuments and a layer or two of cells next to the embryo, which are all that remain of the nucellar tissue. The outer coat of the seed consists of the epidermis and in most cases two layers of subepidermal tissue which is derived from the outer integument; the inner coat consists of two layers, a heavy-walled supporting tissue one cell in thickness and an irregular layer of pigmented cells originating in the inner integument. In addition to these layers and surrounding the embryo are one or more layers of cells remaining from the once many-celled nucellus, most of the nucellar tissue having been absorbed by the embryo.

EXPLANATORY LEGEND FOR FIGURE 7

- A, Embryo in the octant stage. Killed 10 days after pollination. $\times 200$ B, Embryo showing differentiation of dermatogen. Material killed 10 days after pollination. $\times 200$ C, Embryo showing differentiation of plerome and periblem. The first suspensor cell has divided; the daughter cell next to the embryo contributes to the embryonic tissues. Killed 11 days after pollination. $\times 200$ D, Embryo in which the periblem of the root has been completed from the hypophysis. Killed 11 days after pollination. $\times 200$ E, Embryo in which basal daughter cell from first division of the hypophysis has divided longitudinally. $\times 200$ F, Embryo in which hypophysis daughter cell adjacent to embryo has divided by longitudinal walls. $\times 200$ G, Embryo in which the dermatogen of the root tip has been completed. Killed 15 days after pollination. $\times 200$ H, Embryo in which one layer of the rootcap has developed. Killed 17 days after pollination. $\times 200$ I and J, Later stages of embryo development (19 and 21 days after pollination) in which two layers of rootcap have developed. $\times 200$ K, Section through an ovule, showing development of the cotyledons. Pigmented layer differentiating. $\times 40$.

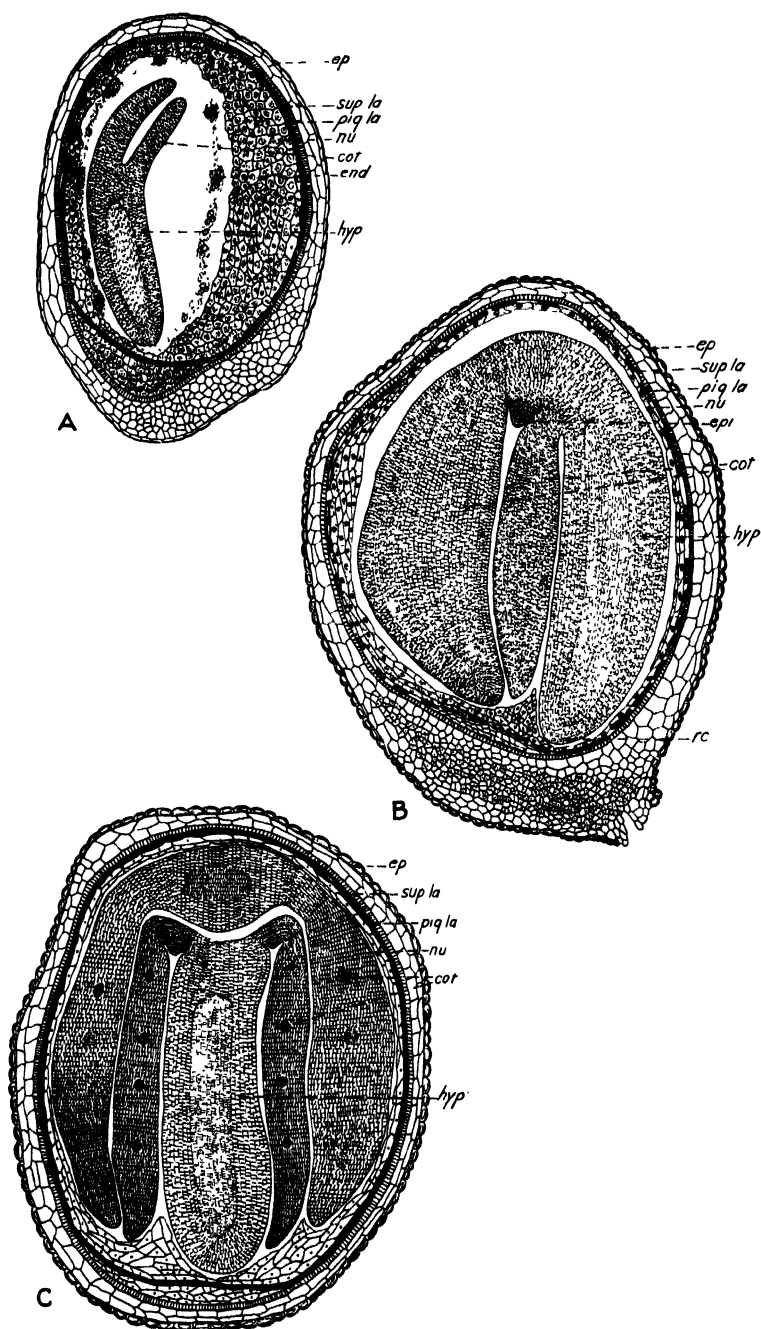


FIG. 8.—A, Section through an ovule, showing development of the hypocotyl. $\times 40$. B, Section through an ovule just before maturity. Cotyledons and hypocotyl well developed; the epicotyl differentiated. $\times 40$. C, Section through a seed near maturity. $\times 40$.

SUMMARY

The differentiation of flower primordia in cabbage varies with the age of the plant and conditions of the environment in which it is placed. Young seedlings overwintered in the open at Washington, D.C., were found to differentiate primordia during the latter part of February or early March. Mature plants that have formed a head differentiate flower primordia soon after cold weather sets in, or about the latter part of October.

The order of appearance of the various cycles of floral organs deviates from the usual acropetalic succession. The four whorls of organs appear in the order of sepals, stamens, carpels, and petals. In this respect they agree with the order reported for *Bursa* (*Capsella*) *bursapastoris* (L.) Brit.

The microsporangia are derived from a single row of hypodermal cells under the epidermis of each lobe of the 4-lobed anther. The primary sporogenous cell divides by a periclinal wall cutting off a primary parietal cell toward the epidermis. The primary parietal layer by periclinal walls divides to form the endothecium, one or more middle layers, and the tapetum. The innermost daughter cell from the first division of the primary sporogenous cell divides to form a plate of pollen mother cells 6 to 8 cells in transverse section. The microsporangia develops before the megasporangia.

The megasporangium is derived from a hypodermal cell of the nucellus. In some cases the archesporium is 2-celled, but only one of these cells functions as a megaspore mother cell. No parietal layer develops in the megasporangium. Two rows of ovules develop in each of the two carpels. The two integuments differentiate almost simultaneously; they appear early in the development of the ovule, develop rapidly, and soon completely enclose the nucellus. The one functioning cell of the archesporium divides to produce a linear row of 4 megaspores, 3 of which fail to develop, disintegrate, and are absorbed by the functioning megaspore, usually the one toward the chalazal end.

The antipodals and synergids are short-lived and are absorbed soon after fertilization.

The time elapsing between pollination and fertilization varies, but fertilization in most cases probably occurs about 5 days after the pollen is applied to the stigma. Numerous ovules were found in which fertilization had apparently not taken place, as indicated by the failure of the embryo sac and the nucellus to develop and the shriveling of the ovules in late stages.

The primary endosperm nucleus divides slightly in advance of the egg. The later divisions follow in rapid succession, as indicated by the many endosperm cells in an ovule found dividing simultaneously. Considerable endosperm develops at the chalazal end of the sac and around the embryo in its early stages. Only a single-celled layer of endosperm lines the major portion of the embryo sac, and this is absorbed by the developing embryo before its maturity.

The larger portion of the mature ovule consists of the embryo. The mature embryo is surrounded by several layers of tissue derived largely from the two integuments. The outer seed coat consists of the epidermis and two layers of subepidermal tissue derived from the outer integument. The inner seed coat consists of a heavy-walled

layer of supporting tissue and a pigmented layer a single cell in thickness just beneath the heavy-walled supporting layer, both of which originate in the inner integument. One or more layers of nucellar tissue may surround the mature embryo. Most of the nucellus is absorbed by the embryo before maturity.

The development of the embryo follows very closely that reported for *Bursa* (*Capsella*). The first transverse division of the embryo cell or proembryo separates the cotyledonary from the hypocotyledonary portions of the embryo. A portion of the embryo is derived from the hypophysis, which is cut off by a transverse wall from the first suspensor cell next to the embryo cell.

THE INHERITANCE OF SOME PLANT COLORS IN CABBAGE¹

By ROY MAGRUDER,² formerly assistant horticulturist, Ohio Agricultural Experiment Station, and C. H. MYERS, professor, Department of Plant Breeding, New York (Cornell) Agricultural Experiment Station

INTRODUCTION

Until recently, only two genetic colors of *Brassica* were recognized, red or purple, and green. In 1924 Kristofferson called attention to a type with colored midribs which probably is the same as the type herein described as sun color. The appearance of a previously undescribed color type called magenta, in the cultures of the junior author immediately aroused interest in its genetic relationship to the previously described color types. The purpose of this paper is to report the results of investigations on the genetic relationships between color types which have been designated as purple, magenta, sun color, and green.

REVIEW OF LITERATURE

As early as 1928 Wiegmann (9)³ reported that red color in cabbage was incompletely dominant over green. E. P. F. Sutton (8), Pease (5), and Allgayer (1) also reported incomplete dominance in the F_1 and concluded that only a single factor was involved in the production of the red color.

In a later paper Pease (6), from his study of a blue kohlrabi $\times$ green savoy cabbage cross, concluded that the purple of blue Vienna kohlrabi was due to two complementary factors. Moldenhawer (4) obtained similar results from a purple kohlrabi $\times$ green brussels sprouts cross.

A. W. Sutton (7) reports plants with purple foliage in the F_2 of Drumhead cabbage $\times$ kohlrabi and Drumhead cabbage $\times$ thousand-headed kale crosses, although no mention was made of color on the F_1 or on either of the parents.

Kristofferson (3, *pt. I*) reports on the inheritance of four color types in crosses between cabbage, brussels sprouts, kale, and broccoli. The type found in red cabbage was described as "dark red violet" throughout all parts of the plant. There were also three types with green blades but differing in the color of the middle vein. "This is of the same dark red violet color as the rest of the leaf in red cabbage,

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³ Reference is made by number (italic) to Literature Cited, p. 247.

light red (violet) in cabbage and brussels sprouts, and green in kale and broccoli."

On the basis of F_1 and F_2 results, he tentatively assumed four factors, A, B, C, and D, to be involved, with the following effects: A produces no color by itself but when present with B the combination causes a dark-red violet coloration of the midrib; B causes a light-red coloration of the midrib; C causes no coloration itself, but with A it produces dark-violet midribs in kale; D causes the general dark-red color of red cabbage.

He experienced considerable difficulty in his classification of midrib colors and in a later report (3, *pt. II*) admits that "for a firm establishing of the factorial basis of the total dark red violet color it may be necessary to grow the F_3 generation."

PLAN OF PROCEDURE

In order to secure one generation per year, seed was sown about the middle of May, the plants were set 18 by 36 inches apart in the field by July 1 and the mature heads harvested about October 15. These were stored at 40° F. until the middle of December, when they were placed in 10-inch pots and grown in the greenhouse at a temperature of 60° to 70°. Flowering occurred during February and March, and seed was mature by May 1.

Glassine bags large enough to inclose the inflorescence were used to prevent any chance cross-pollination that might occur in moving the plants to and from the bench for selfing or crossing. Only the terminal raceme was allowed to develop, as it normally provided sufficient seed if properly pollinated and was much easier to handle without breaking than a plant with several racemes.

Self-pollination was effected by removing an anther or anthers with a pair of curved steel forceps and then touching the anther to the stigmas of open flowers on the same plant. Before working on another plant, the forceps and tips of the fingers were dipped in 95 percent alcohol to kill adhering pollen grains. Flowers to be crossed were emasculated 1 day before they would normally open and were pollinated the following day.

Due to shortage of space in the field, several thousand seedlings were grown in flats in the greenhouse during the summer of 1929. Crowding of plants and high temperature accompanied by periodic water shortage resulted in extreme hardening and a high production of pigment. Readings on these plants were made in September, at which time most of the plants had formed a small rosette and enough of a terminal bud so that purple could be readily distinguished from sun color. Where other data were available to serve as a check, these figures are included in the results even though the proportions of different color types are not exactly like those of the field-grown material.

No attempt was made to classify the field-grown plants for color until after heads had formed, which was usually during September. The color type of each plant was checked on at least two different dates. Plants of the parental lines were grown each year for comparison purposes. The F_2 and some of the other families were grown during two different years.

SOURCE AND DESCRIPTION OF COLOR TYPES

PURPLE TYPE

The purple type is of ancient origin and is represented by several horticultural so-called red varieties. As far as the authors have been able to ascertain from a study of the descriptions given, all the genetic data to date have been concerned with this color, although the word "red" has commonly been used to describe it. All of the American varieties of so-called red cabbage are of this type.

Practically all plants of this color have a heavy "bloom", i.e., are glaucous, which somewhat obscures the undercolor and gives the leaves a dull, gray-purple appearance. Moreover, the presence of chlorophyll in the cells containing the pigments adds to the difficulty of accurately determining the color by the use of any of the available color standards. According to Ridgway,⁴ when examined in diffused light the homozygous purple type with undisturbed bloom may be described as dull dusky purple or naphthalene violet. When the waxy bloom is removed the color most nearly agrees with raisin purple or blackish purple.

The intensity or depth of the purple color increases with the maturity of the plant and is also increased by unfavorable growth conditions such as drought or cold weather. It is present as soon as the seedlings appear above ground although somewhat obscured by the green of the chlorophyll. Purple can be distinguished from magenta with a fair degree of accuracy in the early seedling stages and with almost complete certainty if the young plants are hardened by exposure to low temperatures or drought conditions.

Light is not essential for its formation. It is found throughout the mature head along the midribs and larger veins and more or less diffused between the veins.

When heterozygous for both factors assumed to be responsible for the production of purple, the plant is not so dark a purple as is the homozygous plant, and when the outside leaves of the head are stripped away it can be observed that the purple color is very largely limited to the midribs and larger veins of the leaf. The spaces between veins are white or light green.

MAGENTA TYPE

The term "magenta" was applied by the junior author to a distinct red type in the progeny of a selfed plant of the Danish Round Red variety. Approximately 25 percent of the progeny were magenta, the balance being purple like the parent plant.

With the bloom present the color on the magenta stems and outer leaves matches fairly well with Ridgway's daphne pink or daphne red. In seedlings and heterozygous plants the color is more dilute and varies between light Persian lilac and Persian lilac in tint. The color on the interior of the head where chlorophyll and bloom are practically absent agrees fairly well with Ridgway's spinel red. This type could more properly be called red than the above-described purple type, but to avoid confusion, the term "magenta" has been selected.

⁴ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D.C. 1912.

Like the purple type, the color of this type develops on the stem and leaves inside the cabbage head and is similarly affected by drought and other unfavorable growth conditions. The color is of clearest hue on the bud leaves of young plants or on the leaf base or stem at the base of the mature head. Magenta seedlings may be distinguished when very young from purple ones by holding the young plants between the observer and the source of light or against a sheet of white paper.

In the field, plants that are heterozygous for the magenta factor may on superficial examination be confused with those of the green type because the pink color does not always extend to the tip of the leaf and is obscured by the bloom and chlorophyll color.

In homozygous plants the magenta color is diffused between the veins on the leaves inside the head, whereas in heterozygous plants the color is very largely limited to the midribs and larger veins.

SUN-COLOR TYPE

The term "sun color" was selected for the sun-color type because it develops color only on the portions of the plant exposed to the light. This character is commonly found on horticultural varieties of late cabbage and was isolated in homozygous condition by selling a commercial variety. The color is of the purple type described above and in homozygous plants is found on the exposed midribs, larger veins, along the serrulate or dentate edge of all leaves and diffused between the veins on the outside leaves of the head. It also occurs on the stem when exposed to sunlight. There is very little formation of color in rapidly growing plants of this type and in a normal season it does not develop to the fullest extent until the cool, frosty nights of autumn. Unfavorable growth conditions apparently favor its development even in small seedlings. If the heads mature without encountering cool nights or unfavorable growth conditions, the color may not be sufficiently well developed to permit accurate classification of the plant. In this event, the small heads formed from axillary buds later in the season have been found to provide an accurate index of the plant's color type.

Because of the effect of environmental factors on its expression the sun-color type varies more widely than purple or magenta. In plants heterozygous for this character the color may be limited to a faint purpling of the midrib and the serrulate or dentate margin of the leaves. In other plants from the same family it may be necessary to strip back the outside leaves of the head to distinguish it from heterozygous purple. The leaves and stem of the interior of the head of a sun-color plant are white or very light green, and stripping the head leaves or examining the stem at the base of the head was used as a method of distinguishing it from purple or magenta.

GREEN TYPE

The green type is light green and, except under unusually unfavorable growing conditions, free from any other color on any part of the vegetative plant.

HISTOLOGY OF COLOR DEVELOPMENT

In all the above-described color types, microscopic examination of free-hand sections showed the color (other than green) to be limited to 1 and not more than 2 layers of the nearly round cells of the spongy mesophyll adjacent to the layer of colorless cutinized epidermal cells. The red and purple pigments are contained in the cell sap and are not due to chromoplastids.

EXPERIMENTAL DATA

The probability values in tables 1 and 3 were obtained by direct interpolation from the values given by Fisher (2, *table III*). When the P value is less than 0.05 it is unlikely that the deviations are due to chance alone, and one must look with suspicion on the hypothesis advanced, or account for the deviation in some other way than errors of random sampling.

Although two crosses of an entirely different nature were carried simultaneously, the one involving all the color types will be considered first since it provided all the data necessary for an analysis of the above-described colors.

A cross was made between sun-color and magenta plants and will hereafter be referred to as cross no. 328. The F_1 plants of this cross were all purple, a new type, which at once suggested the interaction of two factors. The F_2 results given in table 1, group 1, show that the parental types were recovered as well as the purple of the F_1 and a pure green type. The distribution of plants in the 4 classes gives a very good fit to the dihybrid ratio of 9:3:3:1, $P=0.86$. Deviations as large as these might therefore be expected through chance alone in about 6 out of 7 trials.

TABLE 1.—*Progenies of succeeding generations from selfed plants of purple, magenta, and sun color found in crosses 328 (magenta $\times$ sun color) and 327 (magenta $\times$ green)*

F₂ PROGENIES OF F₁ PURPLE FROM CROSS 328

Group no.	Number of families	Number of plants				Total number of plants	Calculated ratio	P
		Purple	Magenta	Sun color	Green			
1	2	406	144	134	42	726	9.3 3.1	0.86

F₃ PROGENIES OF F₂ PURPLES FROM CROSS 328

2	28	1,153	384	352	109	1,998	9.3 3.1	0.22
3	6	261	—	78	—	339	3:1	.41
4	3	114	40	—	—	154	3:1	.78
5	6	190	—	—	—	190	All	—

F₄ PROGENIES OF F₃ PURPLES FROM CROSS 328

6	7	85	28	27	13	153	9.3:3:1	0.71
7	1	12	4	—	—	16	3:1	—
8	1	14	—	6	—	20	3:1	.62
9	2	69	—	32	—	101	3:1	.13
10	4	148	—	—	—	148	All	—
11	3	64	24	—	—	88	3:1	.64

TABLE 1.— *Progenies of succeeding generations from selfed plants of purple, magenta, and sun color found in crosses 328 (magenta, × sun color) and 327 (magenta × green)—Continued*F₂ PROGENIES OF F₁ MAGENTAS FROM CROSS 327

Group no	Number of families	Number of plants				Total number of plants	Calculated ratio	P
		Purple	Magenta	Sun color	Green			
12	3		509		192	701	3:1	0.65

F₁ PROGENIES OF F₂ MAGENTAS FROM CROSSES 328 AND 327

13	9		254		75	329	3:1	0.37
14	4		^a 116			116	All	
15	3		50		9	59	3:1	.09
16	6		115			115	All	

F₁ PROGENIES OF F₁ MAGENTAS FROM CROSSES 328 AND 327

17	5		109		41	153	3:1	0.29
18	2		28			28	All	
19	1		4		3	7	3:1	.28
20	1		14			14	All	
21	1		22			22	All	
22	2		34		11	45	3:1	.91
23	2		42			42	All	
24	2		49			49	All	

F₂ PROGENIES OF F₂ SUN COLORS FROM CROSS 328

25	10			^b 272	95	367	3:1	0.70
26	4			224		224	All	

F₁ PROGENIES OF F₁ SUN COLORS FROM CROSS 328

27	2			68		68	All	
28	2			10	4	14	3:1	0.76

^a Plus 8 purple plants^b Plus 1 magenta plant in 1 family, 9 purples in another family, and 6 purples in still another family

One of the F₁ purple plants was back crossed to a pure green plant, and the progeny were distributed in approximately equal numbers in the four color classes as shown in table 3, group 1. The fit of expected to observed on the basis of a 1:1:1:1 distribution is very good, *P* equaling 0.83.

These data seemed sufficient to postulate that two independently inherited factors were involved in the production of the purple color.⁵ The symbol *M* has been assigned to the factor responsible for the production of magenta and *S* to that for the production of sun color.

Because of the labor involved in securing hybrid seed in large numbers, the number of F₁ plants in many test crosses is rather small. It was much easier to secure large numbers of seed by selfing, and

⁵ Kwan, (Chia 'Chi), in a personally published abstract which is filed with the Graduate School of Cornell University, as of June 1933, reports the results of crosses between purple and sun red and purple and green cabbage. The material for the crosses was furnished by the junior author. Kwan found the purple to be incompletely dominant in the F₁ to sun red and concludes that purple is produced by duplicate genes which are cumulative in effect. Using the same purple in a cross with green, he found the F₁ to be intermediate in color and the F₂ to be composed of approximately 9 purple: 3 sun red: 4 green. F₃ results substantiate the hypothesis that the purple and green parents differ by two factor pairs, designated as Gg and Hh. G is a basic factor for pigment which with H gives purple and with h gives sun red. Plants with g are green. It is evident that the purples used differ genetically from those employed in the present study.

more attention has therefore been paid to growing successive filial generations as a test of the genetic nature of the plant than to back crossing it to the double recessive green type or to known testers.

BEHAVIOR OF F_2 COLOR TYPES IN LATER GENERATIONS

PURPLE TYPE

Thirty-six of the purple plants from the F_2 of cross 328 were grown and selfed in the greenhouse. Assuming that purple is due to the action of two independent factors, one would expect to find four genetic types of purples in the F_2 .

The F_3 progeny results given in table 1, groups 2 to 5, inclusive, show that all four expected genotypes were present and the distribution of color types in each group agrees well with the theoretical expectation as indicated by the probability value. Goodness of fit of the proportions of expected genotypes was not calculated as the sample was not a random one, i.e., plants with different degrees of purple pigmentation in the F_2 were selected in proportion to the expected number of each based on a guess as to their genotype.

Selections were made of all the color types present in each of the F_3 groups represented in table 1. The F_4 results from the F_3 purples are given in groups 6-11 inclusive. Families in groups 6, 7, 8 represent selections from group 2, those in groups 9 and 10 from group 3, and those in group 11 from group 4. None of the plants from the pure-breeding group 5 survived to produce progeny. In view of the small number of plants involved it is not surprising that an F_3 plant producing only purple plants should fail to be present in those selected from groups 2 and 4. The distribution of color types in the segregating families was reasonably close to the expected proportions and corroborates the postulated genotype for the F_2 plants.

MAGENTA TYPE

For convenience in presenting the results, the data from the second cross (327) mentioned at the beginning of the section on experimental data, will be included in the results with the magentas from the 328 cross. Cross 327 was made between magenta from the same family as that used in the 328 cross and a green from a family that had been breeding true for this type for several generations.

According to the genotypes postulated, only two kinds of magentas would be present in the F_2 , those (ssMM) which in the F_3 would produce only magenta and those (ssMm) which would produce magenta and green in an approximate 3:1 ratio. Such was the case, as the data in table 1 show. The eight purple plants found in one family may be explained by assuming that a tag was lost from a crossed pod and this pod later harvested with the selfed ones.

The following array shows the relation between the F_4 families and the F_3 families:

- F_3 group 13 represented by F_4 progeny in group (s) 17.
- F_3 group 14 represented by F_4 progeny in group (s) 18.
- F_3 group 2 represented by F_4 progeny in group (s) 19, 20.
- F_3 group 4 represented by F_4 progeny in group (s) 21.
- F_3 group 15 represented by F_4 progeny in group (s) 22, 23.
- F_3 group 16 represented by F_4 progeny in group (s) 24.

In only one case (group 17) did the expected types fail to appear, but in this sample of only 5 plants it is not surprising that both genotypes were not present.

SUN-COLOR TYPE

Fourteen of the F_2 sun colors were selfed and produced an F_3 generation. On the basis of the proposed hypothesis one would expect 2 Ssmm to 1 SSmm. The actual ratio as shown in table 1, groups 25 and 26, is 10:4, a close approximation of that expected. The presence of purple or magenta plants in three of the families was probably due to accidental crosses or to the loss of tags from crossed pods.

The F_4 results given in group 27 of table 1 show that these families, selected from a family in group 26, produced only sun-color plants as expected. The F_3 sun-color plants whose progeny results are shown in group 28 must have been genotypically Ssmm. They were selected from group 2 of table 1.

GREEN TYPE

No difficulty was experienced in separating sun color from green in the F_2 progeny of cross 328 in the fall of 1927 at Wooster. In a culture of the same F_2 grown in the gardens of the Plant Breeding Department, Cornell University, during the same year, the proportion of green plants was much lower than in the Wooster culture and raised the question as to the correctness of the Wooster classification. Nine green plants were accordingly selfed and the F_3 progeny grown in 1928 and 1929.

In 1928 the growing conditions at Wooster were very similar to those of 1927 at Ithaca in that the latter part of the summer was marked by a severe drought accompanied by high temperatures. These conditions were apparently responsible for the development of a small amount of pink color on the midribs of a large number of plants in progeny which were expected to contain only green plants. This color compared in shade more closely with magenta than with sun color and was limited almost exclusively to the middle section of the midrib of the leaves immediately surrounding the head. This type was recorded as questionable sun color. The most intense coloration on sun-color plants, on the other hand, frequently occurred on the upper third of the midrib and along the edge of the leaf. The difference in location and shade of color made it possible to easily separate most of the questionable type from the sun color after these differences were realized. Failure to differentiate accurately between these types in the beginning may account in part for some of the wide deviations from the expected ratios.

The 1929 season was also characterized by hot dry weather during August and the first half of September, which resulted in the formation of pink midribs on many otherwise green plants. Timely and abundant rainfall during the later half of the growing season resulted in rapid growth and the disappearance of this color from some plants. Pease (5) mentions the fact that hot dry weather in England resulted in the formation of light-red or pink color on the leaves of plants that under normal conditions were pure green.

The F_3 results from F_2 greens for each year are given in table 2, group 1, and it will be noted that whereas in 1928 many of the plants

were thought to be sufficiently pigmented to justify a classification of sun color, in 1929 only one plant was so recorded. Part of this shift may have been due to a clearer conception of the differences between sun color and this questionable type as a result of the 1928 experience.

The F_4 progenies (group 7, table 2) of six of these F_3 plants recorded as sun color show that they were incorrectly classified, since none of them produced sun color in the F_4 . Data to be given on intercrosses between F_2 color types support this conclusion.

TABLE 2.—*Progenies of succeeding generations from selfed plants of green, questionable sun color, and sun color from crosses 328 (magenta $\times$ sun color) and 327 (magenta $\times$ green)*

F_3 PROGENIES FROM F_2 GREENS

Group no	Pedigree no	Year grown	Number of plants			Total number of plants
			Sun color	Questionable sun color	Green	
1	328-4-1	1928		9	5	14
	328-4-2	1928		14	1	15
	328-4-2	1929		48	1	49
	328-4-3	1928			3	3
	328-4-4	1928		10	3	14
	328-4-5	1928		7	3	10
	328-4-5	1929		57	2	59
	328-4-6	1928		1	1	2
	328-4-7	1928	26			26
	328-4-7	1929	1	20	10	40
	328-4-8	1928	7		2	9
	328-4-8	1929		10	1	11
	328-11-3	1929		13	6	19
	327-2-1 to 7 inclusive	1929			115	115

F_4 PROGENIES FROM F_3 GREENS

3	328-4-1-3	1929		2	4	6
	328-4-1-8	1929		4	2	6
	328-4-4-10	1929			2	2
	328-4-5-1	1929			5	5
	328-4-8-1	1929		20	1	21
	328-4-8-2	1929		37		38
4	327-2-21-3	1929			25	25
	327-2-21-6	1929			20	20
5	327-2-7-2	1929			14	14
	328-4-15-9	1929			14	14
6	328-4-15-15	1929			9	9

F_4 PROGENIES FROM F_3 PLANTS RECORDED AS SUN COLOR

7	328-4-7-1	1929		5	5	10
	328-4-7-2	1929		1	2	3
	328-4-7-3	1929		3	7	10
	328-4-8-3	1929		27		27
	328-4-8-4	1929		12	1	13
	328-4-8-6	1929		12		12

F_4 PROGENIES FROM F_3 PLANTS RECORDED AS QUESTIONABLE SUN COLOR

8	328-4-1-1	1929		8	6	14
	328-4-1-6	1929		1		1
	328-4-1-10	1929		4	2	6
	328-4-4-4	1929			8	8
9	328-4-5-2	1929		3		3
	328-4-6-2	1929		8		8
	328-4-6-2	1929		1		1
	328-4-24-15	1929		32	6	38

* Plus 3 magenta in each progeny, probably the result of accidental cross-pollination.

The F_4 progeny from F_3 questionable-sun-color plants indicate that this type was not genotypically sun color, since no sun-color plants were produced.

Part of the F_3 green plants again produced plants of the questionable-sun-color type in varying proportions. Evidence presented in the section on intercrosses between F_2 color types indicates that the eight F_2 green plants and the F_3 questionable-sun-color and sun-color plants in their progeny were not genotypically sun color. Because of such evidence the questionable type plants have been classed with the green in all the progeny results to this point.

The appearance of green, questionable sun color, sun color, magenta, and purple in the same progeny discredits the possibility of multiple allelomorphs, and further tests will be necessary to determine whether the appearance of this questionable-sun-color type is due to environmental or modifying genetic factors.

INTERCROSSES OF F_2 COLOR TYPES

As a further check on the genetic constitution of the F_2 plants, crosses were made between the various color types found in this generation. The results are given in tables 3 and 4. Because of the labor involved in hybridization it was not possible to utilize all of the F_2 plants or to make all the possible or desired combinations. It will be noted that those crosses involving green are really back crosses. The genotypes assigned are those suggested by the F_2 or F_3 results.

TABLE 3.— F_1 progenies of backcrosses and intercrosses between F_2 color types of crosses 328 (magenta $\times$ sun color) and 327 (magenta $\times$ green)

F_1 PROGENIES OF F_1 PURPLE OF CROSS 328 CROSSED WITH GREEN OF CROSS 327

Group no.	Families	Genotypes based on F_3 progeny						Number of plants				Total plants	Calculated ratio	P
		Purple	Magenta	Magenta	Sun color	Sun color	Green	Purple	Magenta	Sun color	Green			
1	No 1	SsMm					ssmm	36	20	31	34	No 130	1.1 1:1	0.83

F_1 PROGENIES OF F_2 PURPLES OF CROSS 328 CROSSED WITH GREEN OF CROSS 327

2	13	SsMm					ssmm	158	163		100	188	609 1:1:1:1	<0.01
3	5	SSMm					ssmm	60			68		128 1:1	.48
4	2	SSMm					ssmm	65	73				138 1:1	.50
5	3	SSMM					ssmm	109					109 All	

F_1 PROGENIES OF CROSSES BETWEEN F_2 PURPLES AND SUN COLORS FROM CROSS 328

6	1	SSMM			SSmm			50				50	All.	---
7	3	SSMM			Ssmm			100				100	All.	---
8	2	SSMm			SSmm			23		38		61 1:1		0.05
9	7	SSMm			Ssmm			16		14		30 1:1		.72
10	6	SsMm			SSmm			^b 60		59		119 1:1		.93
11	19	SsMm			Ssmm			54	24	30	7	115 3:1:3:1		<.01

^a Based on results of F_2 generation.

^b Plus 2 magenta and 1 green, probably the result of accidental cross-pollination or self-pollination.

TABLE 3.—*F*₁ progenies of backcrosses and intercrosses between *F*₂ color types of crosses 328 (magenta × sun color) and 327 (magenta × green)—Continued*F*₁ PROGENIES OF CROSSES BETWEEN *F*₂ PURPLES AND MAGENTAS FROM CROSS 328

Group no	Families	Genotypes based on <i>F</i> ₂ progeny						Number of plants				Total plants	Calculated ratio	P
		Purple	Magenta	Magenta	Sun color	Sun color	Green	Purple	Magenta	Sun color	Green			
12	No											No		
13	1	SSMM		ssMm				23				23	All.	
14	1	SSMm		ssMM				9				9	All.	
15	3	SSMm		ssMm				20		12		323	1	0 10
16	7	SSMm		ssMM				167	173			340	1:1	75
16	13	SSMm		ssMm				118	133	43	38	332	3:1:1:1	0 10

*F*₁ PROGENIES OF MAGENTA × GREEN (CROSS 327)

17	1		ssMM				ssmm		50			50	All.	
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*F*₁ PROGENIES OF *F*₁ MAGENTA OF CROSS 327 CROSSED WITH GREEN OF CROSS 327

18	4		ssMm				ssmm		79		79	149	1:1	0 47
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*F*₁ PROGENIES OF *F*₂ MAGENTAS OF CROSS 327 CROSSED WITH GREEN OF CROSS 327

19	1		ssMm				ssmm		14		11	25	1:1	0 56
20	3		ssMM				ssmm		51			51	All.	

*F*₁ PROGENIES OF *F*₂ MAGENTAS OF CROSS 328 CROSSED WITH GREEN OF CROSS 327

21	1		ssMm				ssmm		104		100	204	1:1	0 78
22	2		ssMM				ssmm		52			52	All.	

*F*₁ PROGENIES OF CROSSES BETWEEN *F*₂ MAGENTAS AND SUN COLORS OF CROSS 328

23	1		ssMM		SSmm			13				13	All.	
24	1		ssMm		SSmm			20		32		52	1:1	0 10
25	5		ssMM		ssmm			106	99			205	1:1	.64
26	9		ssMm		SSmm			103	98	53	68	322	1:1:1:1	.01

*F*₁ PROGENIES OF *F*₂ SUN COLOR OF CROSS 328 CROSSED WITH MAGENTA OF CROSS 327

27	4		ssMM		ssmm			30	16			46	1:1	0 01
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*F*₁ PROGENIES OF CROSSES BETWEEN *F*₂ MAGENTAS OF CROSS 328

28	1		ssMM	ssMM					57			57	All.	
29	2		ssMm	ssMm					9		6	15	3:1	0 18

*F*₁ PROGENIES OF *F*₂ SUN COLORS OF CROSS 328 CROSSED WITH GREEN OF CROSS 327

30	5				ssmm		ssmm			103	110	213	1:1	0 65
31	3				SSmm		ssmm			106		106	All.	

*F*₁ PROGENIES OF CROSSES BETWEEN *F*₂ SUN COLORS OF CROSS 328

32	4				SSmm	ssmm				40		40	All.	
33	3				ssmm	ssmm				46	6	52	3:1	0 03

TABLE 4 — F_1 progenies of crosses involving F_2 greens and questionable color types in F_3 from selfed F_2 green plants F_1 OF CROSSES BETWEEN MAGENTA AND F_2 GREENS

Group no.	Female parent	Male parent			Number of plants		
	Phenotype	Pedigree no	Pedigree no	Phenotype	Magenta	Questionable sun color	Green
1.	(Green	328-4-1	243-2-21	Magenta	15		
	do	328-4-2	do	do	6		
	do	328-4-4	do	do	9		
	do	328-4-5	do	do	2		
	do	328-4-6	do	do	27		
	do	328-4-7	do	do	28		
	do	328-4-8	do	do	13		
	do	328-11-3	do	do	12		

 F_1 OF CROSSES BETWEEN MAGENTA AND F_3 TYPES FROM F_2 SELFED GREENS

2.	Questionable sun color	328-4-1-1	243-2-21	Magenta	25		
	do	328-4-1-2	do	do	30		
	Green	328-4-1-3	do	do	21		
	Questionable sun color	328-4-1-4	do	do	13		
	do	328-4-1-6	do	do	18		
	Green	328-4-1-8	do	do	12		
	Questionable sun color	328-4-1-9	do	do	15		
	do	328-4-1-10	do	do	14		
	Green	328-4-4-1	do	do	14		
	do	328-4-4-2	do	do	18		
	Questionable sun color	328-4-4-4	do	do	17		
	do	328-4-4-5	do	do	6		
	do	328-4-4-6	do	do	17		
	do	328-4-4-7	do	do	18		
	do	328-4-4-8	do	do	17		
	do	328-4-4-9	do	do	15		
	Green	328-4-4-10	do	do	21		
	Questionable sun color	328-4-5-2	do	do	23		
	Sun color	328-4-6-2	do	do	20		
	do	328-4-7-1	do	do	15		
3.	do	328-4-7-2	do	do	3		
	do	328-4-7-3	do	do	14		
	Green	328-4-8-1	do	do	13		
	do	328-4-8-12	do	do	11		
	Sun color	328-4-8-3	do	do	7		
	do	328-4-8-4	do	do	3		
	do	328-4-8-6	do	do	8		
	Questionable sun color	328-4-24-15	do	do	13		

 F_1 OF CROSSES BETWEEN F_2 GREENS FROM CROSS 328

3.	(Green	328-4-5	328-4-8	Green	8	
	do	328-4-5	328-4-7	do	1	
	do	328-4-5	328-4-6	do	1	
	do	328-4-6	328-4-8	do	1	3

 F_1 OF CROSSES BETWEEN F_2 GREENS OF CROSS 328 AND GREEN OF CROSS 327

4.	(Green	328-4-1	243-2-5	Green	24
	do	328-4-2	243-2-5	do	22
	do	328-4-3	243-2-5	do	24

 F_1 OF CROSSES BETWEEN F_2 GREENS OF CROSS 327 AND PARENTAL GREEN

5.	(Green	327-2-1	243-2-5	Green	23
	do	327-2-2	243-2-5	do	23

It is noteworthy that in every cross the expected color types were realized, and in the majority of cases the fit of observed to calculated frequencies of the involved color types was very good.

The deviations in group 2 of table 3 from the calculated 1:1:1:1 ratio is large ($P < 0.01$), and practically precludes the possibility of the deviations being due to errors of random sampling. The deviations are greatest in the sun-color and green classes, there being a marked deficiency in the sun color and an excess in the green. An examination of the detailed records shows that more than 50 of the plants listed later as green were recorded originally as questionable sun color and were finally placed in the green class after the results of test crosses with these doubtful plants were known. In this connection it should also be stated that approximately half of the individuals in the larger families were classified on the basis of seedling plants, which may also account for a portion of the error. If, however, the sun-color and green classes are combined, P , calculated on the basis of a 1:1:2 ratio, becomes approximately 0.40. This good fit supports the idea that in this case the error was largely one concerned with classification.

In the case of groups 11 and 26, however, there was a deficiency in both the sun color and green classes. When the seedling cultures were grown in the greenhouse during the summer it was noticed that the purple and magenta plants were the most vigorous when all four types appeared in the same family. It is therefore possible that these smaller shaded plants were more subject to damping off and therefore to proportionately greater losses than the more vigorous purple or magenta plants. There may also have been some differential viability of the embryos.

In order to determine whether the F_2 plants classified as green were really green or light sun color as suggested by the F_3 data, they were crossed with magenta. If a mistake in classification had been made and the plants were really homozygous or heterozygous for the sun-color factor, the F_1 of a cross with magenta should be purple or approximately 50 percent purple, depending upon the genetic constitution of the sun-color plant. If it was green the F_1 should be uniformly magenta. The data in table 4, group 1, indicate that the eight F_2 plants tested were not sun color, since none of the back crosses contained any purple plants.

It will be recalled in the discussion of the F_3 results from selfed F_2 greens, that in addition to the expected green type some F_3 plants were sufficiently pigmented to apparently warrant being classified as sun color, others were called questionable sun color, and still others green. In order to check on the correctness of the classification, plants of all three types were crossed with the parental magenta.

The F_1 progeny results are given in table 4, group 2. None of the sun-color or questionable sun-color plants produced any purple plants in the F_1 and are therefore assumed not to be sun color. The pigmentation present was probably of a physiological nature due to the unfavorable growth conditions. These data corroborate the F_4 results obtained from the selfed F_3 plants, discussed in a previous section, which showed that none of the F_3 plants from F_2 greens produced sun color, magenta, or purple in the F_4 .

The F_2 green plants were very poor producers of seed, and in all crosses where they were used as pistillate parents the number of progeny is accordingly small.

Four crosses were made between F_2 greens, and the F_1 progeny were either green or of the questionable sun-color type. Tests with similar questionable plants have shown (group 2 in table 4) that they were not genetically sun color.

Three of the F_2 green plants from cross 328 were back crossed to the 243-2-5 green, and all the 70 progeny were green, as shown in table 4, group 4.

Two green F_2 plants from the 327 cross were also crossed with the parental green and produced F_2 progenies of 46 green plants (table 4, group 5).

These results corroborate those of the F_3 and F_4 and furnish further proof of the correctness of the postulated genetic interrelations of the described color types.⁶

DISCUSSION

In attempting to interpret the results of previous work on the inheritance of cabbage color, in terms of the present report, one is confronted with the task of first solving the problem of color nomenclature. The absence of a standardized nomenclature makes it impossible to determine whether such terms as "red", "wine red", or "dark red violet" refer to the purple or magenta type as herein described. Pease placed "red" cabbage and "blue" kohlrabi under the common term "purple" which indicates their synonymy in Europe. It is also probable that sun color was not separated from pure green.

The clear-cut 3:1 segregations of red and green in the F_2 generation of crosses between red (purple) and green of Sutton, Pease, and Allgayer are probably best explained by assuming that the cross was made between purple and sun color, the classification being made on the basis of the color of the blade and neglecting the colored midrib of the sun-color type. It is also possible that the counts may have been made before the sun color developed sufficiently to become noticeable. Allgayer's description, however, agrees well with the writers' observations on the grades of color in an F_2 of a magenta $\times$ green cross, although it is possible that a summer record may reveal similar grades in the F_2 progeny of a sun color $\times$ purple cross.

Magenta crossed with green would also give a 3:1 segregation of colored to green. If the cross were made between purple and pure green it would be necessary to group purple and magenta together in the red class, and sun color and green in the green class to get a 3:1 ratio in the F_2 generation. It hardly seems possible that magenta should fail to be distinguished from purple, if present.

If it is assumed that Kristofferson's light-red-vein type is identical with the writers' sun color, and that the "dark red violet throughout" is the same as the writers' purple, then the results of his crosses of

⁶ In a personal letter dated July 26, 1931, J. C. Miller, Louisiana State University, Baton Rouge, La., gives some results of a cross between an annual-seeding magenta from the junior author's cultures and an inbred line of the Jersey Wakefield variety which conforms to the sun-color type herein described. The F_1 plants were all purple and the F_2 contained approximately 9 purple:3 magenta:3 sun red:1 green. F_3 data are not yet available. Miller found that the best time to detect the difference between sun red and green was during the months of December and January. His results corroborate those reported in the present paper.

light-red vein with green, with light-red vein and with dark-red violet throughout, agree with those in the present study.

If the green kale was really pure green, the results of the cross between it and dark-red violet cabbage do not agree with the writers' results from a purple $\times$ green cross. Kristofferson reports the F_2 as composed of approximately "3 dark red violet leaved plants to 1 green leaved plant." The veins of the green F_2 plants from the cross of dark-red violet $\times$ green kale were reported as being green. The same green kale crossed with light-red-veined cabbage produced an F_2 generation comprised of approximately 9 dark-red violet:3 light red:4 green vein, so it apparently brought in genetic factors not concerned in Kristofferson's above-mentioned results.

It is to be expected that other modifying factors or allelomorphs will be discovered as the above-described color types of *Brassica oleracea*, var. *capitata* are used in crosses with other varieties of this species or with other strains of this botanical variety.

SUMMARY

This paper reports the results of inheritance studies with purple, magenta, sun color, and green plant colors of cabbage, *Brassica oleracea*, var. *capitata*.

Data are presented on crosses between magenta with green and sun color, back crosses of the F_1 and F_2 color types with the double recessive green, behavior of F_2 color types in the F_3 and F_4 generations, and progenies of intercrosses between F_2 color types.

The results are interpreted as supporting the following genetic formulas for the respective color phenotypes used in this study:

SM = purple

sM = magenta

Sm = sun color

sm = green

The existence of all possible genotypes of these phenotypes has been demonstrated in the course of this work.

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THE COMPOSITION OF FEEDSTUFFS IN RELATION TO NUTRITIONAL ANEMIA IN CATTLE¹

By W. M. NEAL, *associate in animal nutrition*, and R. B. BECKER, *specialist in dairy husbandry, Florida Agricultural Experiment Station*²

INTRODUCTION

Cattle suffer from a nutritional anemia, locally called "salt sick," on certain definite areas of the coastal plains. Affected animals recover when moved to certain other ranges but become salt sick again within a few months if returned to the original range. Cattle are moved from range to range in this manner to enable the owners to utilize the pastures and to keep their cattle healthy.

In cooperative work with owners of affected cattle (7)³, this nutritional anemia was corrected by feeding supplements of iron, or of iron and copper, without other changes in feeding or management, thus enabling owners to make maximum use of affected lands without shifting their cattle.

It was noted from a field survey (7) that the affected ranges consisted of white and gray sands, cumulose muck lands, and shallow residual soils overlying marl. Healthy ranges were on red and yellow sands, clays, shallow sands underlaid with clay, and soils subject to overflow from clay lands.

Forage and grain samples were collected at the time of the cooperative work and field survey in order to determine quantitative differences in the composition of such samples from the various areas. Analyses of the feedstuffs are reported in this paper.

LITERATURE REVIEW

A deficiency of iron has been found in the natural rations of livestock in several parts of the world. Aston (3, 4) demonstrated this deficiency to be the cause of the condition known as "bush sickness," or "skinnies" in sheep and cattle in parts of the counties of Rotorua, Matamata, and Tauranga in the North Island, New Zealand. Orr (20) and Gunn (12) have shown that "pining" among the sheep in the Cheviot Hills in the south of Scotland, and "nakurutis" in the Nakuru district of Kenya Colony are both associated with this deficiency. Dickinson (9) has verified Aston's prediction that "coasty disease" on King Island, Tasmania, is of the same etiology. The condition found in Florida (7, 17, 19) is complicated in certain areas by a concurrent deficiency of copper.

Aston and his associates (5, 6), and Askew and Riggs (1) made comparative analyses of forages from affected and healthy areas in New Zealand and found definite differences in composition of the crops from the two areas, which will be discussed later in relation to the data presented.

¹ Received for publication Jan. 26, 1933; issued August 1933.

² The assistance of J. R. Greenman and William T. Dunn with parts of the analytical work herein presented is acknowledged.

³ Reference is made by number (italic) to Literature Cited, p. 254.

The evidence of iron deficiency in other areas consists of soil analyses from the healthy and affected areas, and the prevention and correction of the condition in sheep and cattle by administration of iron compounds.

METHODS OF INVESTIGATION

As soon as salt sick in cattle had been demonstrated to be a nutritional anemia caused by a lack of iron, or of iron and copper (7, 17), the collection of a series of feed samples from healthy and affected areas was instituted. Designation of such areas was made according to statements of cattle owners based on their experience, and upon direct observations of cattle on these areas. In some few places, the deficiency was so extreme that the landholders had abandoned any further attempt to handle cattle. In many other places, few attempts were made to raise young stock.

Wire grass (*Aristida* species, mostly *A. stricta*) was selected as the forage to indicate whether or not the degree of deficiency on the various ranges, and the relation of stage of growth and season of the year to the incidence of the condition, could be measured analytically. This particular grass was selected because of its predominance on the majority of the range areas. Other forages were collected incidental to the investigation.

Samples gathered to show the effect of stage of growth were always taken from unfertilized land and on sites not subject to overflow during the rainy season. Successive samples were taken from identical areas. Whenever possible, adjacent samples were taken on burned and unburned range. This was done because of the practice of "burning the woods" (8), by turpentine operators to protect their cups from uncontrolled or accidental burning, and by cattlemen to provide succulent new growth in the late winter and early spring resulting from burning off the "rough." Wire grass was gathered by pulling the individual blades. Only living grass was taken, representative of the growth on the land.

Corn was selected to represent the grains, as it is the only grain grown extensively in Florida. Velvetbean samples were secured whenever possible. This latter crop generally is harvested by pasturing in the fall and winter, when the grasses are short or old. Some cattlemen believe that it aids in a natural recovery from nutritional anemia. This belief may be due to the fact that the velvetbeans usually are grown on the more fertile or fertilized lands, thus having a higher content of the mineral elements.

Particular care was exercised to avoid soil contamination, as only a small amount of clay soil adhering to the plants would lead to a large error in an iron analysis. This error is greatest in the case of low-growing forages such as lespedeza, and ensiled crops where handling in the field is unavoidable. After drying, the samples were ground in a clean Wiley mill. Any iron contamination from this source should be nearly uniform for all samples.⁴

Analyses made included calcium, magnesium, and phosphorus by the method of Morris, Nelson, and Palmer (16), iron by a modification of the method of Kennedy (15), and proximate analyses of some of the crops more or less peculiar to Florida by the methods of the Association of Official Agricultural Chemists (2).

⁴ See footnote on opposite page.

RESULTS

Gradation was observed as to the quality of the various ranges. On some it is impossible to maintain cattle; on others the condition of salt sick never occurs, while on the marginal areas cattle do not develop salt sick, nor do affected cattle placed on them make recovery. Forage samples from marginal ranges have been omitted from this study.

Wire grass is the principal forage on the open ranges other than the muck lands of this region. It makes some growth during the entire year, but the proportion of older growth and dead grass increases progressively. During the winter this is sufficient to interfere with easy grazing, and hence parts of the range are burned over periodically. Wire grass, being a bunch grass, withstands this burning and immediately puts out a succulent new growth.

The comparison of the mineral content of samples of wire grass from both healthy and affected areas that have been burned over, or left rough, is presented in table 1, together with a comparison with other feed crops.

The wire-grass samples from the healthy range areas show a uniformly higher content of all the minerals determined than do those from the affected areas, in general amounting to approximately 20 percent. The difference between burned and unburned areas is greatest in the early spring and almost disappears by July. The seasonal trend on both burned and unburned areas is toward a decreased mineral content, a late sample from a healthy area possibly showing a lower mineral content than does an early sample from an affected area.

Other forages produced on affected lands had a greater percentage of all the mineral elements except iron than did the wire grass. The legumes were very much higher in calcium and phosphorus. The significantly higher percentages of iron in lespedeza and in soybean-silage samples may be attributed in part to soil contamination. The lespedeza was of a low decumbent growth. Slight soil contamination was visible in the silage samples. Differences due to soil contamination would tend to magnify themselves between healthy and affected soils because of the higher iron content of the healthy soils associated with the increase in the clay fraction.

⁴ Since this manuscript was prepared, the following analyses have been made to determine the extent of iron contamination from the Wiley mill. There is some increase in the iron content due to grinding in the Wiley mill. The differences between areas reported, considering that averages of a number of samples have been given, remain significant.

Description of sample	Iron content of sample	
	Before grinding	After grinding
Wire grass	Percent	Percent
Sample A	0.0194	0.0222
Sample B	.0213	.0197
Sample C	.0199	.0288
Sample D	.0174	.0206
Sample E	.0213	.0230
Average	.0196	.0228
Velvetbean seed	.0105	.0146
Velvetbean pods	.0084	.0161
Corn, grain	.0083	.0172

TABLE 1.—*Mineral content of the dry matter of various feedstuffs in relation to nutritional anemia in cattle*

Feedstuff and month cut	FORAGES									
	Samples from healthy areas					Samples from salt-sick areas				
	Samples	Ca	Mg	P	Fe	Samples	Ca	Mg	P	Fe
	Number	Percent	Percent	Percent	Percent	Number	Percent	Percent	Percent	Percent
Wire grass (burned area)										
March	6	0.230	0.178	0.276	0.0256	7	0.153	0.132	0.221	0.0191
April	6	.221	.167	.231	.0233	7	.177	.151	.208	.0184
May	6	.181	.122	.169	.0240	7	.140	.100	.144	.0194
June	6	.167	.110	.122	.0207	7	.129	.090	.117	.0172
July	6	.142	.095	.106	.0172	7	.117	.078	.106	.0143
Total or average	30	.188	.134	.181	.0222	35	.143	.110	.159	.0177
Wire grass (unburned area)										
March	2	.262	.096	.209	.0196	2	.153	.117	.108	.0165
April	2	.193	.107	.168	.0217	2	.167	.148	.164	.0182
May	2	.190	.108	.171	.0209	2	.114	.086	.102	.0155
June	2	.200	.077	.125	.0177	2	.107	.086	.102	.0218
July	2	.203	.086	.118	.0164	2	.107	.074	.096	.0120
Total or average	10	.210	.095	.158	.0193	10	.127	.102	.114	.0168
Alfalfa hay, no. 1 ¹	9	1.173	.164	.251	.0214					
Beggarweed	2	1.158	.272	.293	.0181					
Corn silage						9	.274	.179	.291	.0191
Lespedeza	6	1.086	.227	.240	.0617	2	1.114	.479	.286	.0165
Natal grass	1	.508	.247	.330	.0385	2	.489	.313	.310	.0130
Soybeans	15	1.083	.365	.406	.0255					
Soybean silage	23	1.178	.369	.406	.0402					
GRAINS AND BY-PRODUCTS										
Cornmeal	6	0.016	0.122	0.291	0.0128	9	0.011	0.120	0.332	0.0094
Velvetbean seed	6	.160	.184	.451	.0110	3	.138	.180	.421	.0102
Velvetbean feed meal	6	.291	.205	.335	.0113	3	.243	.206	.322	.0113
Cottonseed meal, 36 percent protein	2	.235	.975	.362	.0196					
Cottonseed meal, 43 percent protein	3	.251	1.001	.375	.0134					

¹ Purchased on the Kansas City market in 1929, 1930, and 1931.

Corn meal (from shelled grain) showed a higher iron content from the healthy areas, while no difference was evident between the velvetbean samples analyzed. Cottonseed meal milled in Alabama and Georgia from seed grown on red clay soils had a higher iron content than did either the corn or velvetbeans. The 36 percent protein grade of meal with its higher proportion of hulls had a higher iron content than had the 43 percent meal. Cottonseed meal is considered one of the curative feeds. Proximate analyses of some of the above samples are presented in table 2.

The higher crude-protein content of the wire grass from burned ranges, and the remarkably constant percentage of crude fiber regardless of stage of growth of this plant are particularly significant. It would seem to be a desirable forage in the earlier stages of growth, as has been proven by experience. The carbohydrate content would seem to be adequate at any season.

The lespedeza samples were collected during the bloom stage, when the growth was desirable for pasturage. The soybean silage was made from soybeans in an earlier stage of growth than those reported elsewhere (18). The protein content of the velvetbeans was higher than in those summarized by Henry and Morrison (14).

TABLE 2.— Proximate analyses of some of the feedstuffs listed in table 1

Feedstuff and month cut	Samples	Moisture	On moisture-free basis				
			Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract
	Number	Percent	Percent	Percent	Percent	Percent	Percent
Wire grass (burned area)							
March	7		5.08	10.70	34.01	48.79	1.41
April	7		5.24	9.28	34.40	49.76	1.34
May	7		3.92	6.52	35.03	52.98	1.55
June	7		3.12	4.46	36.21	54.69	1.52
July	7		3.23	3.60	36.90	54.63	1.64
Wire grass (unburned area)							
March	3		3.60	4.26	33.35	56.99	1.80
April	3		3.66	4.64	34.72	55.41	1.67
May	3		3.25	4.49	35.00	55.59	1.67
June	3		2.64	3.90	36.83	55.07	1.56
July	3		2.81	3.73	37.40	54.53	1.53
Beggarweed	2	8.98	4.89	15.78	36.88	40.23	2.23
Lespedeza	7	9.66	5.86	14.93	26.33	50.71	2.17
Soybeans, green	15	74.91	9.34	12.00	36.32	39.74	2.55
Soybean silage	23	75.43	10.70	9.04	40.51	36.54	3.20
Velvetbean seed	9	11.08	3.25	27.07	7.29	56.64	5.10
Velvetbean feed meal	9	11.35	3.66	20.95	13.79	57.30	4.30

DISCUSSION

Differences in mineral contents of wire grass from burned and unburned areas were greater than those observed by Hart, Guilbert, and Goss (13) in alfalfarai and wild oats. Grazing animals preferred the forage on the burned range, as Camp (8) also has pointed out. The analyses herein presented explain to some extent why cattle should make better growth and be more thrifty on such ranges. However, this report makes no attempt to evaluate the practice of burning the range, either for a single season or over a number of years.

The new grass on a burned area was of a more recent growth and had a higher mineral and protein content than had grass taken from adjacent unburned areas. This difference incident to stage of growth and season of the year, has been observed with other pasture plants (1, 6, 10, 13, 20, 21). Most of the samples included in these reports represent the new growth after clipping, whereas the Florida samples represent the entire accumulation of growth before the time of sampling, except as reduced by incidental grazing. The results reported are therefore more nearly due to stage of growth than to the season of the year.

The fact that the poverty of iron, rather than any of the other minerals that parallel it, was the factor limiting the nutritive value of the wire grass, was proven in cooperative feeding trials with cattle on affected ranges (7, 19). A supplement of bonemeal, without any change in feeding practice, did not lead to improvement in condition of salt-sick cattle on affected wire-grass ranges, while the administration of an iron-and-copper supplement was effective. The use of other forages grown on affected soils, with their higher content of the mineral elements other than iron, did not alleviate the condition.

Whether or not animals can utilize the iron contained in soil contamination (of the forages) is unknown. If they can, then the difference between the iron intake of cattle on the two types of range would be greater than is indicated by analyses of the feedstuffs. Askew and Riggs (1) have offered this explanation to account for finding an iron content of 0.008 percent from bush-sick range forage,

and of 0.009 and 0.011 percent from healthy range forages at Glenhope, New Zealand. These percentages are lower than those presented herein. However, all New Zealand analyses were discarded if there were any indications, whatever, of soil contamination. They represent season averages.

Aston (3, 4) gave the iron percentages on plant ash from healthy and affected areas. The differences observed were verified in a later report (5) which included the percentage of iron in the dry matter. Red clover, white clover, and crowfoot grass had an iron content in the dry matter of 0.0077, 0.0084, and 0.0098 percent, respectively, when grown on bush-sick soils, and 0.0147, 0.0119, and 0.0119 percent respectively, when grown on healthy soils. Samples from marginal areas had an iron content between these values.

Godden and Grimmett (11) found differences in the amount of iron in the dry matter of oats and mustard grown in pot cultures, using soils from pining and bush-sick lands, in comparison with healthy soils.

The differences observed between the forages of healthy and affected areas in the various parts of the world are comparable, even though the absolute concentrations vary. This may be due to differences in methods of analysis. All the evidence reported supports the results secured by the feeding of supplements to the livestock in the regions under consideration.

SUMMARY AND CONCLUSIONS

The percentage of calcium, magnesium, phosphorus, and iron tend to decrease with advancing stage of growth in wire grass, this tendency being more marked on the burned than on the unburned range areas in Florida. A similar trend is noted in the crude protein content of this grass. These differences are associated with stage of growth, and practically disappear by midsummer.

Wire grass grown on salt-sick range areas contained less iron, calcium, magnesium, and phosphorus than did that from areas where cattle are not affected with this nutritional anemia. Other forages and grains analyzed also followed this trend.

These differences support the results obtained by use of iron-containing supplements, with livestock on ranges where the condition called salt sick occurs, and are in harmony with investigations in other known iron-deficient regions.

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THE FLIGHT RANGE OF THE HONEYBEE¹

By JOHN E. ECKERT²

Formerly, *associate apiculturist, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION AND REVIEW OF LITERATURE

The economic value of the honeybee depends, for the most part, upon its ability to pollinate flowers and to produce a surplus of honey and beeswax. In selecting a proper location for an apiary, therefore, one must consider not only the flora, climate, topography, and the nature of the soil, but also the distances bees must fly in quest of food. With respect to the last factor, a review of the available literature has failed to reveal much pertinent information.

As early as 1844 Huish (5, p. 419-429)³ reviewed the writings of Huber and others and gave an account of his own experiences in contrast to the opinions then current concerning the distances bees fly in search of nectar and pollen. He stated that some of his bees flew 3 miles to hills richly covered with heath and that he knew of an instance in which bees flew at least 4 miles over water to gather nectar on an island. Other investigators of that period were of varying opinions on this question, giving distances of from 1 to 4 miles, according to Huish. It is apparent from his writings, however, that the distances given were merely estimated and thus subject to considerable error.

Waite (11) in 1898 advised that it was necessary to have sufficient bees at least within 2 or 3 miles of an orchard to effect the proper pollination of pomaceous fruits. Hutson (7) found under New Jersey conditions that for best results colonies should be scattered within an orchard at the rate of approximately one colony to the acre.

Miller (8, p. 148), in Illinois, considered that if the distance was greater than 2 miles the quantity of honey would be reduced. Hutchinson (6), in Michigan, thought that bees would range for 2.5 miles in all directions from a given apiary, while Dadant (3, p. 65) wrote that as a general rule bees (near Hamilton, Ill.) made their harvest of honey

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² The writer is indebted particularly to H. Rauchfuss, A. D. Hardy, E. O. Rauchfuss, F. G. Rauchfuss, and G. H. Sechrist for their cooperation, and to Aven Nelson, professor of botany and president emeritus at the University of Wyoming, for the identification of the plants collected in the areas in which the experiments were conducted.

³ Reference is made by number (italic) to Literature Cited, p. 285.

within a radius of 2 km (1.25 miles). Dadant also stated that he believed bees would fly much farther in following a small valley, or when working on flowers along a road, than in passing above a forest or a hill. Buttel-Reepen (2, p. 162) found that bees would orient themselves within a circle of 3 to 4 km (1.86 to 2.48 miles), and where nectar was available at a greater distance from the apiary, with no other source intervening, they would fly 5, 6, and even 7 km (3.11 to 4.35 miles), and under exceptional conditions 12 km (7.46 miles).

One of the most concrete reports reviewed by the writer on the flight range of the honeybee was that of Gowland (9, p. 54). Gowland found that the seed yield of fields of sweetclover located beside an apiary was much heavier than that of any other field except one three eighths of a mile away. A field 4.5 miles from the apiary had a very light yield of seed. In the same report he states: "* * * the bees seemed to concentrate their efforts on certain fields rather than to take the fields in order according to distance from their hive." Gowland thought that this might be one reason why the yield of seed did not decrease directly with an increase in distance.

A considerable difference is also found in laws intended to regulate the location of apiaries. A New Zealand law (1), for example, requires that beekeepers keep their colonies at least 5 miles from the fruit-drying centers, while a Utah law (4) makes it unlawful for migratory beekeepers to locate their apiaries within 2 miles of an established apiary. The same Utah law also requires that all hospital yards (for the treatment of diseased colonies) be located at least 2 miles from other apiaries.

It is reasonable to suppose that the differences in opinion as to the effective flight range of the honeybees may be due to a certain extent to the effect of the locations on the distances the bees had to fly, or they may have been due to a lack of careful observation or to a combination of the two factors. The experiences of such able beekeepers as Miller, Dadant, Hutchinson, and many others have led them to believe that the flight range of the honeybee varies somewhat according to the physical factors of different locations.

The present paper reports the results of experiments conducted in Wyoming and Colorado on the flight range of honeybees and their distribution within a nectar-producing area. The experiments were conducted during the summers of 1927, 1928, 1929, and 1930 and may be divided into three phases. The first observations were made on the flight of bees from colonies located on a prairie during a dearth of nectar when an artificial source of food was provided. In the second series observations were made on colonies located on a stretch of badlands lying between two irrigated districts supporting good growths of alfalfa and sweetclover, and in the third, experiments were conducted with apiaries located within sweetclover territory.

FLIGHT OF HONEYBEES TO AN ARTIFICIAL SOURCE OF FOOD

The first attempt to determine the flight range of the honeybee was made on a wide stretch of prairie west of Laramie, Wyo. The location seemed an excellent one in which to determine the distances bees would fly to an artificial source of food when no important natural source was available.

The Big Hollow, essentially a wind-blown formation about 9 miles long by 3 miles wide and so called because its floor lies between 100 and 150 feet below the level of the surrounding country, contains several fresh-water and alkaline lakes and several ridges (fig. 1). The floor of the formation, which is fairly level to rolling with the exception of the ridges, is covered with grasses, small clumps of cacti, and other vegetation kept short by a lack of moisture and is devoted largely to the grazing of range animals. At the time of the experiment there were no trees or even sagebrush to break the landscape. Only five widely scattered, abandoned homesteads were located in the hollow, and but a small portion of the entire area was under fence.

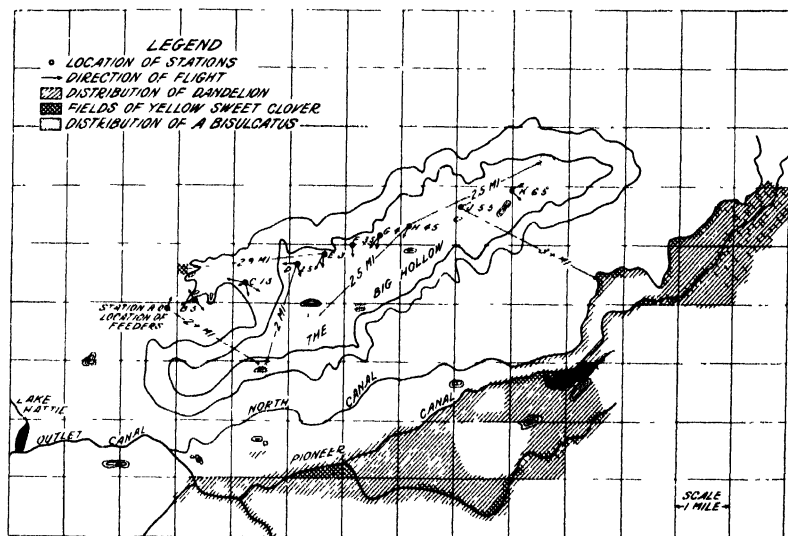


FIGURE 1 —The Big Hollow, west of Laramie, Wyo., where the first attempts were made to determine the flight range of the honeybee

Numerous wild flowers were present during the period of the experiment, but they were of little importance except for furnishing pollen. Certain members of the genus *Astragalus* which covered small areas in the vicinity of the lakes were visited by the bees for pollen and possibly a little nectar. Near the west end of The Big Hollow 3 acres had been planted to yellow sweetclover several years before, but at the time of the experiment the sweetclover covered only about three fourths of an acre. This field came into bloom near the close of the observations. Other than this one field of sweetclover, the nearest nectar-producing plants, dandelion and sweetclover, were at least 3 miles from the center of The Big Hollow.

EQUIPMENT AND METHODS

On June 1, 1927, twenty 1-story colonies of Italian bees of approximately equal strength were moved into The Big Hollow and distributed at 10 stations which extended in a straight line for 6.5 miles (fig. 1). In the western portion of The Big Hollow the first station, A-0, was located. From this point the line of the stations extended

northeasterly away from the direction of the prevailing winds with the colonies faced toward station A-0. A small shack and a few outbuildings at station A-0 were used as temporary headquarters.

Two feeders (fig. 2) were placed in the open at station A-0 and were kept filled with a sirup made of 20 pounds of granulated sugar and 5 pounds of sweetclover honey dissolved in 75 pounds of water. This solution was colored light red with a vegetable dye found to be non-injurious and nonrepellent to the honeybee.

In order to determine from what stations the bees came or to what stations they returned after their visits to the feeders, the majority of

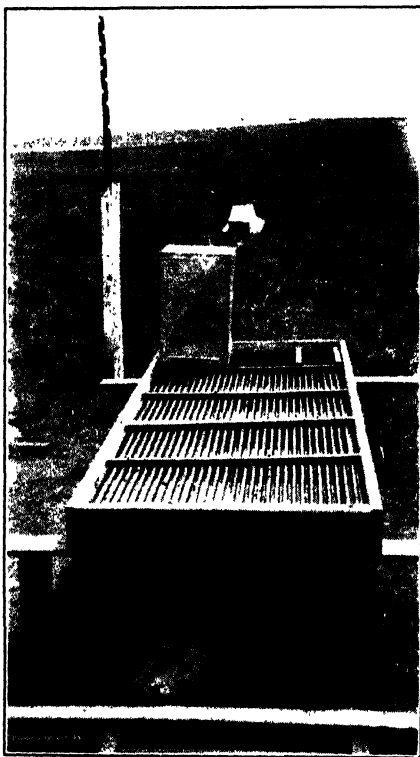


FIGURE 2—Type of feeder for honeybees used in The Big Hollow.

ees in one colony at each station and most of the bees that came to the feeders were marked with distinctive colors. The various colors were applied to the bees at the stations in the form of a fine spray as they crawled up the sides of a box into which they had been shaken. The coloring materials consisted of suspensions of paint pigments in shellac and ethyl alcohol, 1 part shellac and 1 part pigment, by weight, to 4 parts of alcohol. Thus the bees in one colony at station B-0.5⁴ were marked with burnt umber, those at station C-1.5 with Indian red, etc. Most of the bees that visited the feeders were marked individually with vermilion.

Although spraying the bees with the alcohol-shellac mixture apparently had no lasting harmful effect upon them, it did not prove a success, as but small quantities of the pigments adhered to their bodies. Some of the bees were well marked, but they were too few to be of much

use in determining the flight from any one station or in finding the marked bees in the field. Several hundred bees in each colony were later marked individually, and the pigments adhered to them for many days.

In order to determine whether the bees had procured an appreciable quantity of sirup or any other food, whenever the weather conditions permitted each colony was weighed before flight started in the morning and again after flight had stopped for the day.

Records were kept of the general climatic conditions during the period of the experiment.

⁴ The numbers after the station letters refer to the distance in miles each station was located from the feeders.

At each station pollen was collected from returning bees at various intervals for later determination.

In many instances when bees were discovered on plants in the field, they were marked in order that they could be identified later at their hives. When bees so marked in the field were found in their hives, the distance between the hive and the area in which they were marked was recorded.

The hives at each station were searched, generally near the close of each day, for bees marked in the field or at the feeders to determine if any sirup was being carried.

RESULTS

The bees at station A-0 flew to the feeders within a few hours after they had been set out and obtained a significant quantity of sirup during the period of the experiment. The bees at station B-0.5 were at the feeders on the second day after the sirup had been made available. The colonies at that station, however, did not accumulate enough sirup during the entire time of the experiment to show an increase in weight, although the loss was less than that of colonies at the other stations. The bees of only one colony at station C-1.5 found the feeders and then only after some of the field bees had been carried to the feeders where they were fed, marked, and released. The day after this was done, other bees from the same colony also found the feeders. The colonies at station C-1.5 lost weight during the period of the experiment, but the average loss in weight was less than that recorded at the more distant stations (table 1). No bees located beyond the 1.5-mile station were ever seen at the feeders.

TABLE 1.—*The average net gain or loss in weight, by stations, of the colonies in The Big Hollow, June 5 to July 10, 1927, when sugar sirup was used as a source of food*

Station	Gain (+) or loss (—) in weight	Station	Gain (+) or loss (—) in weight
	<i>Kilograms</i>		<i>Kilograms</i>
A-0	+2 33	F-3 5	—5 27
B-0 5	- 1 48	G-4	—5 48
C-1 5	- 3 40	H-4 5	—4 04
D-2 5	—5 49	J-5 5	—3 51
E-3	—5 48	K-6 5	

^a Queenless

Several attempts were made to induce the bees to fly farther than 1.5 miles by baiting them with sirup-filled tins which were moved by short stages in the direction of the feeders. No bees could be made to follow these tins for more than 0.7 mile. This may have been due to the general absence of landmarks, as in one instance it was noted that a marked bee, bearing pollen, made a mistake of one-half mile in the location of its own hive. An automobile used in making the rounds to the stations had stopped at the station from which the marked bee came before moving on to the next station, one-half mile distant. The bee evidently included the automobile among the landmarks when it left for the field and in returning flew to the station at which the automobile had stopped.

Bees were traced for at least 3 miles from several stations and were found carrying certain pollens that were available only outside of The Big Hollow.

Near the close of the experiment the field of yellow sweetclover near station A-0 came into bloom, as did also the sweetclover growing to the east and south of the line of colonies. The bees deserted the feeders for these fields and carried sweetclover pollen to all stations but F-3.5. The two colonies at this station were queenless during the greater part of the experiment. Several hundred bees were marked as they worked in the sweetclover near station A-0. Bees thus marked were found at stations A-0, B-0.5, C-1.5, D-2.5, and E-3, but at none of the other stations. The bees at the stations beyond E-3, with the exception of F-3.5, had to fly out of The Big Hollow, a distance of at least 3 miles, for the sweetclover pollen they obtained.

TABLE 2. -Weather conditions at station A-0 in The Big Hollow in 1927

Date	Wind				Temperature			Character of sky
	Average velocity	Prevailing direction	Maximum velocity	Direction of maximum velocity	Highest	Lowest	Average	
	Miles per hour		Miles per hour		° F	° F	° F	
June 4	8 8	NE	16 0	N	80	61	71 1	Partly cloudy
June 5	11 7	NW	17 5	W	57	47	58 9	Do
June 6	3 0	NW	13 0	NE	67	47	59 9	Do
June 7	10 1	NW	21 5	N	79	48	68 5	Do
June 8	17 6	SW	30 5	SW	81	53	72 7	Do
June 9	16 5	W	28 5	NW	72	58	67 7	Clear
June 10	13 6	SW	22 5	S	82	48	69 9	Partly cloudy
June 11 *	9 5	SW	23 5	SE	74	50	63 7	Do
June 12	13 0	SE	19 0	E	52	43	48 2	Do
June 13	11 0	SE	13 8	SE	59	41	50 2	Do
June 14	5 4	SE	14 0	NE	68	42	55 1	Cloudy
June 15	16 0	N	28 0	N	53	48	49 4	Do
June 16	11 4	NW	24 5	NW	68	37	59 0	Partly cloudy
June 17	17 9	W	34 5	W	74	49	61 2	Do
June 18	10 7	S	23 5	NW	77	55	66 3	Do
June 19	8 0	SW	35 5	W	76	50	61 3	Do
June 20	20 2	N	26 5	N	65	54	61 5	Clear
June 21	16 7	N	26 0	N	62	49	55 5	Partly cloudy
June 22	2 7	(?)	16 0	S	70	40	61 8	Clear
June 23	22 7	W	32 5	SW	84	66	78 9	Do
June 24	21 7	W	32 5	W	87	69	80 5	Do
June 25	18 1	SE	30 5	SE	86	55	72 2	Do
June 26	19 3	SW	30 5	SW	86	68	79 8	Partly cloudy
June 27	15 3	SW	24 5	SW	79	63	70 2	Do
June 28	6 6	SE	13 0	NW	66	58	60 5	Do
June 29	13 6	SW	41 0	SW	74	55	63 6	Do
June 30	18 2	NW	28 5	W	65	49	58 5	Do
July 1	10 6	SW	23 5	W	75	47	65 9	Clear
July 2	11 7	SW	28 0	SW	80	54	72 3	Do
July 3	15 8	SW	27 0	SW	80	60	72 4	Partly cloudy
July 4	16 8	SW	37 0	W	81	59	67 3	Do

* Last observations taken at 5 30 pm.

° No observations made between 8 a.m. and 12.30 p.m.

The combs in the colonies at stations G-4, H-4.5, J-5.5, and K-6.5 showed that the bees obtained nectar and pollen apparently from the dandelion which grew abundantly at distances of 3 and 4 miles from these stations. The colonies at stations J-5.5 and K-6.5 obtained enough nectar from dandelion and sweetclover to minimize losses in weight.

The weather conditions (table 2) that prevailed during the period of the experiment were not altogether favorable to bee flight. The

comparatively low temperatures, together with the large number of cloudy and windy days, tended to retard the activities of the bees. It was evident, however, that the bees flew farther for pollen and nectar than they did for sirup. Whether or not bees will fly more than 1.5 miles to an artificial source of food in locations containing more landmarks was not ascertained.

FLIGHT OF HONEYBEES TO A NATURAL SOURCE OF FOOD

LOCATION

The second phase of these experiments was conducted in northwestern Wyoming in the summers of 1927, 1928, and 1929, on two irrigated areas separated by at least 17 miles of badlands (fig. 3).

The general contour of the country between the two irrigated areas is comparatively level for the first 5 miles from its northern border and then becomes rolling to rough until the bench land at the south is reached. Drainage is toward the Shoshone River on the north, and the slope of the land is such that parts of the green fields and the trees bordering the river are visible from the badlands at a distance of several miles. In fact, both irrigated areas can be seen from many points along the old winding stagecoach road that connects the two.

The creeks in the badlands (fig. 3) were dry during the summer months except for short periods after rains and were marked by wide flood plains overgrown with denser vegetation than that which grew on the higher land.

The summer vegetation in this area consisted, for the most part, of small clumps of cacti (*Opuntia fragilis*), stunted sagebrush (*Artemisia tridentata*), some greasewood (*Sarcobatus* sp.), an admixture of the saltbushes (*Atriplex*), rabbitbrush (*Chrysothamnus*), which grew along the stream beds, and a scattering of various grasses, sedges, and dead stalks of spring-flowering plants. The small composite *Machaeranthera tanacetifolia* grew in abundance on the badlands in 1927 and 1928, and its color added a dash of blue to the otherwise gray aspect (fig. 4). Only a few gnarled and twisted cottonwoods were found along the banks of Coon and Whistle Creeks. The only plants from which bees could secure nectar were confined to the irrigated areas. Numerous prairie-dog mounds and large ant hills, the latter in the center of cleared spaces several feet in diameter, and a double row of telephone poles which crossed the badlands between the irrigated areas may have served as landmarks for the bees in their flights over this semidesert land. The irrigated areas, on the other hand, were known to be good beekeeping territory, and a number of commercial apiaries were located there.

EQUIPMENT AND METHODS

As it was evident that the badlands could provide no appreciable source of nectar for bees during the period of the experiments, the general plan of procedure called for the placing of colonies on the badlands at various distances from the irrigated area bordering the Shoshone River. The bees would thus be compelled to fly to the irrigated areas for nectar, and it was thought that records of the gains or losses in weight of the colonies would indicate the effect of distance upon their activities in the matter of honey production. The

fact that they actually flew to the irrigated areas was checked further by the identification of the pollens they carried.

The colonies used in the experiments in 1927 and 1928 were taken from apiaries located near Powell, Wyo., and as far as possible were of uniform strength in adult bees and brood. Each colony used in 1929 consisted of 4 pounds of bees and 4 frames of brood taken from

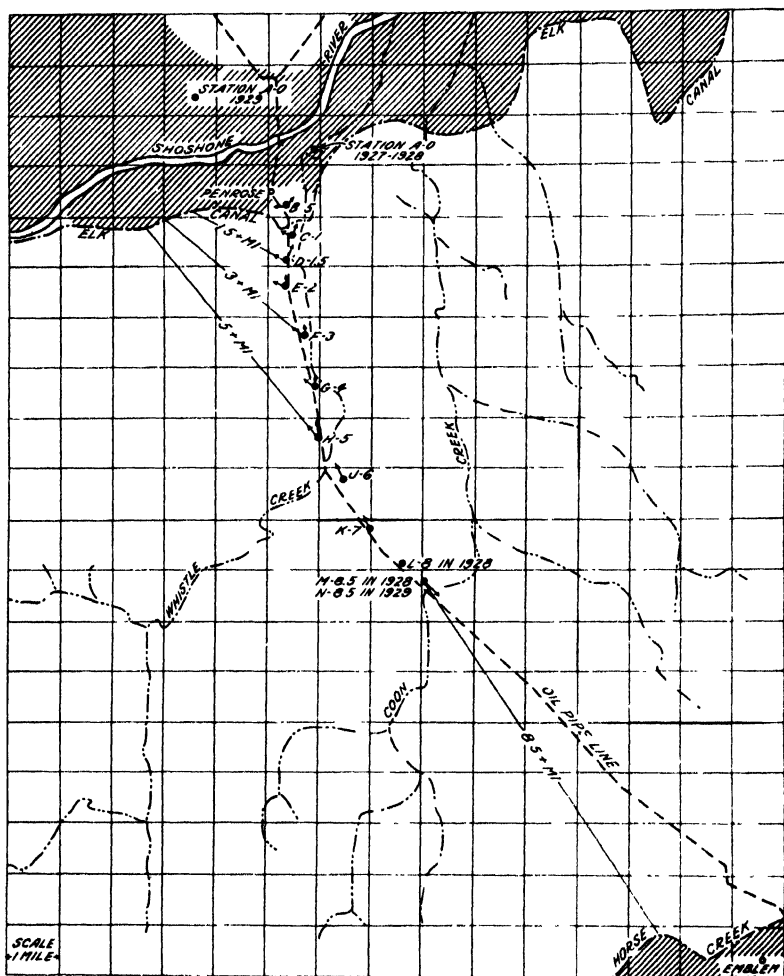


FIGURE 3.—A diagram of the district east of Powell, Wyo., in which the honeybee-flight experiments were conducted in 1927, 1928, and 1929. The shaded areas represent the irrigated areas in which fields of alfalfa and sweetclover were located. The badlands lie between them. Colonies were located at the stations numbered and the directions of flight are indicated by arrows.

overwintered colonies. An attempt was made to secure the 4 pounds of bees and a queen from 1 colony for each colony formed. In several instances, however, bees had to be obtained from two or more colonies in order to secure the 4 pounds. In such cases laying queens were introduced into the newly formed colonies. Equal numbers of Caucasian and Italian colonies were included in each of the 3 years. The

colonies used in 1927 and 1928 were somewhat stronger than those used in 1929.

The colonies were placed in practically the same locations each year, although certain changes in the number of stations were made during 1929, as will be described later.

The stations, each consisting of several colonies, were located with the aid of a map issued by the United States Reclamation Service (10). The distances between the stations and certain points in the irrigated area bordering the Shoshone River were checked either by an automobile odometer or by surveying instruments. Consequently the distances given in this report represent the minimum distances the bees had to fly on quiet days. On windy days these distances would be increased somewhat, especially for the bees located at the stations beyond 5 miles, as on such days the bees flew close to the ground and often had to deviate from a straight line.

In 1927 three colonies were placed at each of the first 10 stations (fig. 3). The first group of colonies, at station A-0, was 0.5 mile

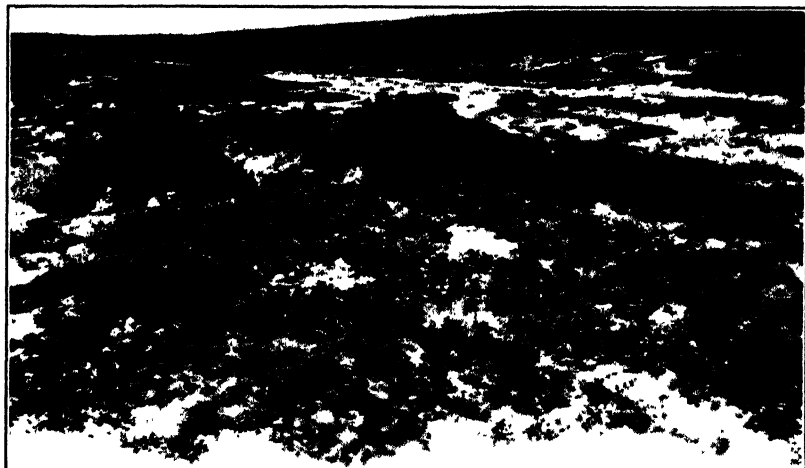


FIGURE 4. - A typical spot on the badlands, showing the characteristic vegetation.

within the irrigated area. The other 9 stations were on the badlands in a line extending practically at right angles to the irrigated area along the Shoshone River, the last station (K-7) being 7 miles from a source of nectar.

In 1928, 5 colonies were placed at each of the first 2 stations, 4 at station C-1, and 3 at each of the other 7 stations. After the first week one colony was moved from station B-0.5 and another from C-1 to the positions indicated as L-8 and M-8.5, respectively.

Forty-two colonies were used in 1929. Station A-0 was placed farther within the irrigated area in what was considered an even better location than it occupied in 1927 and 1928; it contained 4 colonies, 2 Caucasian and 2 Italian. The next 9 stations, which occupied the same positions as during the 2 previous years, also contained 2 Italian and 2 Caucasian colonies each. The eleventh station, N-8.5, contained 1 Caucasian and 1 Italian colony. After observations had been made for 13 days, the colonies at stations K-7, J-6, H-5, and G-4

were moved to stations closer to the source of nectar. A second shift of colonies was made after another 13-day period, at which time the colonies at stations C-1, E-2, and F-3 were concentrated at stations A-0, B-0.5, and D-1.5.

None of the colonies were manipulated more than was necessary to provide supers or stores as needed, and all were examined alike on the same days. Colonies more than 4 miles from the irrigated areas were supplied with honey. There was no swarming during the 3 years, although some colonies became queenless. The records of the queenless colonies have not been included in the data.

All hives at the first 10 stations were weighed each evening after the bees had stopped flying, unless weather and road conditions prohibited. When the observations were omitted on any day during the period of the experiment, the figure recorded for the following day represented the net loss or gain for 2 days, so that this figure will be larger than when only 1 day's loss or gain is included. Generally, there was less than an hour's difference between the time the first and the last colony was weighed. In 1928 it was impracticable to weigh the colonies at stations J-6 and K-7 as often as the other colonies because of rains which made Whistle Creek impassable. The colonies at these two stations and those at stations L-8 and M-8.5 were moved across this creek before the termination of the experiment.

OBSERVATIONS AS TO DISTANCE OF FLIGHT

The bees began to carry in pollen within a few hours after they were placed at the stations. The fact that the pollen carried was sweet clover indicated that the bees flew from 0.5 mile to 7 miles in 1927 and at least 8.5 miles in 1928 and 1929. As these figures represent the minimum distances from the nectar source, additional flight was necessary for the bees to obtain a load of pollen or nectar. In all probability the bees at the 8.5-mile station flew at least 18 or 20 miles on each round trip.

All bees within 7 miles of the irrigated area bordering the Shoshone River flew north, while those at the 8.5-mile station flew to the irrigated area in the opposite direction. The nectar sources at the south were approximately 0.1 mile closer to the station 8.5 miles out than were the nectar sources at the north. No bees were observed to return to the 8.5-mile station from the north.

The direction of flight of the bees from any one station was generally over a narrow angle and in one particular direction, although some bees flew in other directions as well.

EFFECT OF DISTANCE OF FLIGHT UPON THE GAIN OR LOSS IN WEIGHT OF COLONIES

EXPERIMENTS IN 1927

All the normal colonies at stations within 4 miles of the irrigated area increased in weight. Colonies beyond the 4-mile point lost weight. The average gain in weight of the colonies at station A-0, located within the source of nectar, was somewhat less than that of the colonies 0.5 mile out on the badlands. From the 0.5-mile station the average gain in weight per station decreased gradually with an increase in distance, until a loss was recorded at the station 5 miles out, and the average loss became greater at stations J-6 and K-7, respectively (table 3 and fig. 5).

TABLE 3.—Daily average net gain (+) or loss (—) in weight of the colonies at the different stations, August 1927

Station	Col- onies	Average gain or loss per colony, in kilograms, on August —																Total of daily average net gains or losses in weight period	Average age daily net gain or loss in weight for 18- day period
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	17	18	
	Num- ber																		Kilo- gram
A-0-----	2	-0.25	+0.29	+1.81	+1.34	+1.30	+0.11	+0.12	+1.50	+1.67	-0.37	+2.08	-0.08	+0.17	+0.21	+0.80	+0.77	+0.01	+11.48
B-0.5-----	3	-0.14	+0.33	+2.40	+1.95	+1.81	+0.30	+0.17	+1.83	+1.87	-0.06	+2.05	-0.17	+0.48	+0.10	+0.58	+0.95	-0.08	+0.637
C-1-----	3	-0.14	+0.16	+1.74	+1.08	+1.36	-0.17	+0.12	+1.18	+1.55	-0.16	+2.05	-0.72	+0.30	+0.06	+0.52	+0.89	+0.15	+7.98
D-1.5-----	3	-0.34	+0.09	+2.00	+1.64	+1.62	-0.14	-0.12	+1.19	-1.30	-0.15	+1.89	-0.76	+0.22	-0.07	+0.44	+0.67	+0.10	+10.57
E-2-----	3	-0.02	-0.14	+0.87	+1.64	+1.42	-0.17	-0.24	+1.08	-1.36	-0.24	+1.98	-0.54	+0.27	+0.23	+0.36	+0.84	-0.06	+5.87
F-3-----	3	-0.10	-0.20	-0.01	+1.68	+2.00	-0.82	-0.53	+1.24	+1.38	-0.31	+1.77	-0.92	+0.05	+0.19	+0.30	+0.70	+0.15	+9.66
G-4-----	3	-0.18	-0.08	-0.51	+0.31	+1.88	-0.54	-0.62	+0.79	+1.91	-0.31	+1.20	-0.74	+0.13	+0.05	-0.05	+0.51	-0.07	+8.24
H-5-----	3	-0.15	-0.20	-0.48	-0.43	+0.71	-0.47	-0.49	+0.24	+0.52	-0.32	+0.75	-0.74	+0.12	+0.06	+0.18	+0.18	+0.18	+2.36
J-6-----	3	-0.18	-0.14	-0.37	-0.49	-0.24	-0.24	-0.35	+0.09	+0.24	-0.34	+0.56	-0.75	+0.08	-0.02	-0.24	+0.00	-0.14	+0.077
K-7-----	3	-0.04	-0.04	-0.24	-0.45	-0.43	-0.40	-0.26	-0.19	-0.20	-0.28	+0.30	-0.57	+0.07	+0.02	-0.10	-0.30	-0.20	-3.91

* No records were obtained for Aug 15, but those obtained on Aug 16 include the net loss or gain for both Aug 15 and 16

Colonies at all stations lost weight on the first day after they had been placed in position. On the second day the colonies at the stations up to and including station D-1.5 gained weight. A gain was made at the 2-mile station on the third day. Stations F-3 and G-4 showed the first gain on the fourth day, and station H-5 made a gain on the fifth day. The colonies 6 miles away from the source of nectar made a small gain on the ninth day, but thereafter the total losses were greater than the gains. The colonies at the 7-mile station made a slight gain in weight on the thirteenth day, but as in the case of the colonies at stations H-5 and J-6 the average total losses were greater thereafter than the gains (table 3).

From the standpoint of honey production, 1927 was considered a poor year by the beekeepers operating in the territory where these experiments were conducted.

EXPERIMENTS IN 1928

Inasmuch as the colonies at the 5-, 6-, and 7-mile stations lost weight during 1927, each colony in 1928 was given a shallow super of honey as a reserve food chamber, but these had to be removed within a few days and replaced with supers of foundation in order to provide room for the nectar that the bees were bringing in. The food chambers were left on the colonies at stations L-8 and M-8.5.

The colonies at each of the first 10 stations made material gains. The average daily gain or loss in weight per colony was again less at the station within the nectar-producing area than at some of the stations out on the badlands. The effect of distance was less pronounced in 1928 than it was in 1927. In 1928 a distance of 3 to 5 miles showed little effect on the average daily gain or loss in weight, although the average gain was greater for the colonies at C-1, D-1.5, E-2, and F-3 than for those at the other stations. The average data for this period show that the colonies 0.5 to 2 miles away from the irrigated area made greater gains than the colonies within the area. Beyond the 3-mile point a gradual decrease in weight roughly proportional to the increase in distance was recorded. The average daily gain, however, of the colonies 7 miles from the source of nectar in 1928 closely approximated that of the colonies within the irrigated area in 1927 (fig. 3 and tables 3 and 4).

TABLE 4.—Daily average net gain (+) or loss (—) in weight of the colonies at the different stations, July 1928

Station	Colo- nies	Average gain or loss per colony, in kilograms, on July—														Total of daily aver- age net gains or losses in weight 21-day period	Average daily net gain or loss in weight for 21-day period	
		3	5	8	10	12	14	15	17	19	20	21	22	23	24	25		
A-0	Number	5															Kilograms	
B-0.5	4	+0.30	+1.14	+0.87	+1.06	+2.39	+2.32	+0.92	+1.15	+3.49	+0.48	+1.80	+1.96	+1.63	+1.95	+1.33	+0.987	
C-1	3	+0.38	+1.11	+0.90	+1.78	+2.53	+2.51	+1.38	+1.23	+3.52	+0.60	+2.08	+2.80	+2.68	+1.67	+1.06	+1.000	
D-1.5	3	—0.7	+1.13	+1.16	+1.09	+2.89	+3.23	+1.08	+1.63	+3.17	+1.04	+2.79	+2.22	+2.09	+2.50	+1.14	+1.080	
E-2	3	—0.78	+0.84	+1.02	+1.74	+2.70	+3.45	+1.16	+1.31	+2.80	+1.04	+2.09	+2.22	+2.14	+1.90	+1.23	+1.132	
F-3	3	0.48	+0.43	+0.36	+0.80	+3.07	+3.13	+1.63	+1.46	+2.13	+0.63	+1.96	+1.42	+1.43	+1.87	+1.02	+1.090	
G-4	3	0.00	+0.53	+0.28	+1.40	+3.61	+3.69	+1.69	+1.55	+1.16	+1.13	+2.15	+1.45	+1.83	+1.91	+1.77	+0.761	
H-5	3	—0.13	+0.73	+1.31	+1.14	+2.89	+2.75	+1.19	+1.45	—	—	—	—	+3.83	—	—	+0.664	
I-6	3	+0.02	+1.01	+0.64	+1.60	—	—	—	+6.45	—	—	—	—	+3.47	—	—	+0.584	
K-7	3	—0.33	+0.79	—	+1.60	—	—	—	+6.74	—	—	—	—	+3.47	—	—	+0.584	

The colonies at stations J-6 and K-7 were weighed at irregular intervals, the last weights being taken on July 23. The average was therefore for a 21-day period.

The colonies at stations A-0 and B-0.5 made an average gain in weight the first day, while all the other colonies lost weight, with the exception of those at F-3, which showed no change, and at J-6, where there was a slight gain. The colonies were not weighed again until the third day, by which time all had increased in weight (table 4).

Beekkeepers in the vicinity considered honey production to be above the average in 1928.

EXPERIMENTS IN 1929

FIRST PERIOD

During the first part of the experiment in 1929 (July 3 to 15) the colonies were in practically the same positions as in 1927 and 1928, except that station A-0 was moved to what was thought to be a more favorable location within the irrigated area, and only one station, N-8.5, was situated beyond K-7.

The colonies on the badlands at stations B-0.5, C-1, D-1.5, and E-2 again made greater average gains in weight than the colonies at station A-0, which was within the irrigated area. Beyond the 2-mile point the adverse effect of distance was increasingly apparent, until losses were recorded at stations J-6 and K-7, as well as at N-8.5 (table 5).

TABLE 5.—*Daily average net gain (+) or loss (—) in weight of the colonies at the different stations during the first period, July 3 to 15, 1929*

Station	Col- onies	Average gain or loss per colony, in kilograms, on July —									Total of daily average net gains or losses in weight	Average daily net gain or loss in weight for 13-day period
		3	4	6	9	10	11	12	14	15		
	Num- ber										Kilo- grams	Kilo- grams
A-0.	3	+0 85	+1 03	+2 39	+1 41	+1 34	+0 61	+1 86	+1 36	+0 64	+11 49	+0 884
B-0 5	4	+1 14	+1 24	+3 06	+1 73	+1 33	+ 64	+1 75	+1 55	+ 82	+13 26	+1 020
C-1	4	+1 38	+1 38	+2 86	+2 30	+ 90	+ 41	+1 54	+1 47	+ 37	+12 61	+ 970
D-1.5	4	+1 48	+1 55	+3 25	+1 83	+ 94	+ 21	+1 59	+1 32	+ 21	+12 38	+ 952
E-2	4	+1 53	+1 56	+3 50	+1 31	+ 91	+ 26	+1 63	+1 15	+ 15	+12 00	+ 923
F-3	3	+1 05	+1 17	+3 41	+ 93	+ 60	— 11	+1 34	+1 13	+ 03	+9 55	+ 735
G-4	4	+ 18	+ 43	+1 63	+ 44	+ 26	— 13	+ 78	+ 61	— 07	+4 13	+ 318
H-5	3	— 13	+ 03	+ 84	+ 08	+ 10	— 24	+ 45	+ 52	— 18	+1 47	+ 113
J-6	4	— 18	— 26	— 29	— 43	+ 10	— 33	— 02	+ 05	— 21	— 1 57	— 121
K-7	4	— 09	— 39	— 43	— 26	— 14	— 17	— 27	— 16	— 12	— 1 97	— 151
N-8.5	2										— 1 75	— 134

* 2 of the 5 colonies originally located at station A-0 became queenless during this period and, being abnormal, their production was not recorded.

In this period the colonies were not weighed until the second day after they had been placed on location. By that time all colonies up to and including those at station G-4 gained weight. On the following day the colonies at the 5-mile station gained weight while those at the more distant stations lost weight. On the ninth day the colonies at station J-6 gained weight. (This coincided with the data of colonies located at the same station in 1927.) The colonies at stations K-7 and N-8.5 did not gain weight during any day of this period.

SECOND PERIOD

In order to demonstrate more clearly the effect of length of flight on the change in weight of certain of the colonies moved, on July 16 some of the colonies from stations G-4, H-5, J-6, and K-7 were moved to stations A-0, B-0.5, D-1.5, E-2, and F-3, as indicated in table 6. One colony (no. 20) was moved from station E-2 to station A-0. This increased the number of colonies under observation at points which seemed to be the most important from the standpoint of practical honey production.

TABLE 6. — *Changes made in the location of colonies at the beginning of the second period, July 17 to 29, 1929, and a comparison between the gains (+) or losses (—) in weight of the colonies in their new locations and in their old locations during the first period*

Colony no.	Old location	New location	Distance moved	Average daily net gain or loss in weight	
				At old location	At new location
			Miles	Kilograms	Kilograms
20	E-2	A-0	4 0	+1.116	+1.460
37	K-7	A-0	9 4	— 154	+1 599
39	K-7	A-0	9 4	— 152	+1 593
27	G-4	B-0 5	3 5	+ 462	+1 006
34	J-6	B-0 5	5 5	— 096	+1 412
36	J-6	B-0 5	5 5	— 079	+ 940
26	G-4	D-1 5	2 5	+ 214	+ 892
29	H-5	D-1 5	3 5	+ 050	+1.235
40	K-7	D-1 5	6 5	— 147	+1.459
25	G-4	E-2	2 0	+ 375	+ 672
28	G-4	E-2	2 0	+ 220	+ 516
33	J-6	E-2	4 0	— 142	+ 569
35	J-6	E-2	4 0	— 166	+ 619
30	H-5	F-3	2 0	+ 065	+ 806
31	H-5	F-3	2 0	+ 225	+ 336
38	K-7	F-3	4 0	— 153	+1 140

During the forenoon of the day after the colonies were moved, several hundred bees were flying around the spot where station J-6 had been located. Caucasian and Italian bees were present in approximately equal numbers, and most of them were carrying sweet-clover pollen. As two of the colonies from station J-6 had been moved at least 4 miles to station E-2, it was evident that some of the field bees, on leaving their hives on the following morning, had flown to the irrigated area, obtained pollen, then returned over the old route to which they had been accustomed, and in so doing covered a minimum distance of 8 miles.

A larger number of bees, most of them bearing sweetclover pollen, were found flying around the place where station H-5 had been. Two of the colonies from this station had been moved 2 miles to station F-3, and one had been moved 3.5 miles to station D-1.5. Still more bees were found clustered on sagebrush or flying around the spot where station G-4 had been; most of them were also carrying sweet-clover pollen. Two of the colonies from this station had been moved 2 miles, another 2.5 miles, and the fourth colony 3.5 miles. In gathering pollen and in returning to their old locations, these bees had flown a minimum of 4.5 to 6 miles. In all probability some of the bees from all the colonies moved, with the exception of those moved from

station K-7, returned to their former locations, thus flying minimum distances of from 6.5 to 8 miles.

The bees that returned to the old locations were allowed to enter supers and were taken back to the new locations the same evening. On the second day after the colonies had been moved, bees were flying around the old locations at stations J-6, H-5, and G-4, and they were again returned to their hives. On the following day only a few bees were found flying around the old location of G-4. It is well known that field bees sometimes remain out overnight and return to their hives in the morning. The fact that no bees were found flying around the former location of station K-7 indicates that the field bees did not generally stay out the night the colonies were moved.

During the second period the colonies at all the stations collected sweetclover pollen; those at the first six stations made appreciable gains; while those at station N-8.5 lost weight (table 7). A distance of 1 mile evidently did not appreciably reduce the gain of the colonies, but beyond that point the reduction in gain increased with the distance of flight. This apparent effect of distance may have been accentuated by the fact that more of the field bees from the colonies moved to stations E-2 and F-3 may have been lost than was probably true of the colonies moved to the other station.

TABLE 7.—Daily average net gain (+) or loss (—) in weight of the colonies at the different stations during the second period, July 17 to 29, 1929

Station	Colo- nies	Average gain or loss per colony, in kilograms, on July—											Total of daily aver- age gains or losses in weight	Average daily gain or loss in weight for 13-day pe- riod
		17	18	19	20	21	22	23	24	25	26	27	28	29
A-0.....	7	+1.39	+2.11	+0.99	+1.03	+1.07	+1.46	+1.54	+1.51	+0.90	+0.44	+1.82	+1.48	+1.21
B-0.5.....	7	+1.71	+2.39	+1.21	+1.84	+2.12	+1.60	+1.55	+1.89	+1.71	+0.53	+2.37	+2.06	+1.400
C-1.....	4	+1.57	+2.27	+1.22	+1.42	+2.11	+1.75	+1.22	+1.84	+1.40	+0.51	+2.04	+1.79	+1.345
D-1.5.....	7	+1.53	+1.74	+0.88	+2.33	+1.82	+1.92	+1.13	+1.91	+1.11	+0.47	+2.36	+1.91	+1.253
E-2.....	7	+0.90	+0.97	+0.58	+0.03	+1.19	+1.63	+0.76	+1.21	+0.00	+0.12	+1.87	+1.71	+0.832
F-3.....	6	+0.89	+1.01	+0.41	+0.11	+1.00	+1.68	+0.53	+1.13	+0.03	+0.15	+1.89	+1.94	+0.784
N-8.5.....	2												+1.74	+1.134

* No records taken on July 18.

* One of the colonies at this station which became queenless during the first period was made queen-right, resumed normal activities, and was thereafter included in the record.

The effect of moving the colonies nearer to the source of nectar, as shown in table 6, was quite striking in some instances. The greatest difference occurred in those colonies that had been moved from 6 and 7 miles from the source of nectar to within the irrigated area or 1.5 miles from it. These colonies had lost weight during the first period of 1929 but gained when placed in the new locations. In every instance colonies moved nearer to the source of nectar made greater gains than they had in their former positions (table 6) and in several instances they showed greater gains than the colonies already located at the stations to which they had been moved (table 9). These gains were made in spite of the loss of field bees due to the change in locations. It was evident that the colonies at the more distant stations lost weight during the previous period because of the distances that separated them from the source of nectar rather than on account of any insufficiency in the number of field bees.

THIRD PERIOD

The colonies at stations C-1, E-2, and F-3 were moved to stations A-0, B-0.5, and D-1.5, respectively, on the evening of July 29, as indicated in table 8. The same procedure was followed as that used during the previous period, although even greater precautions were taken to see that the bees reoriented themselves in the new locations.

TABLE 8.—Changes made in the locations of colonies at the beginning of the third period, July 31 to Aug. 9, 1929, and a comparison between the gains (+) or losses (—) in weight of the colonies in their new locations and in their old locations during the second period

Colony no	Old location	New location	Distance moved	Average daily net gain or loss in weight		
				At old location	At new location	
			Miles	Kilograms	Kilograms	
9	C-1	A-0	3 6	+1 436	+1 325	
10	C-1	A-0	3 6	+ 888	+ 820	
11	C-1	A-0	3 6	+1 398	+ 654	
12	C-1	A-0	3 6	+1 658	+1 358	
41	N-8 5	A-0	10 75	— 152	+1 824	
42	N-8 5	A-0	10 75	— 115	+1 863	
17	E-2	B-0 5	1 5	+1 656	+1 547	
18	E-2	B-0 5	1 5	+ 806	+1 281	
19	E-2	B-0 5	1 5	+1 006	+1 386	
25	E-2	B-0 5	1 5	+ 672	+ 883	
28	E-2	B-0 5	1 5	+ 516	+ 749	
33	E-2	B-0 5	1 5	+ 569	+1 160	
35	E-2	B-0 5	1 5	+ 619	+ 908	
21	F-3	D-1 5	1 5	+ 692	+ 825	
22	F-3	D-1 5	1 5	+ 626	+ 613	
23	F-3	D-1 5	1 5	+ 705	+ 728	
24	F-3	D-1 5	1 5	+ 735	+1 174	
30	F-3	D-1 5	1 5	+ 806	+1 064	
31	F-3	D-1 5	1 5	+ 336	+ 512	

Soon after noon of the next day, which was bright and clear, many bees had returned to their old location (station F-3), and by evening 3.4 pounds of bees (approximately 14,000 bees) had collected in the hive placed there to trap them. Most of these bees were loaded with sweetclover pollen and nectar. The colonies from this station had been moved 1.5 miles closer to the source of nectar. Only about one

sixth as many bees from station E-2, which was also moved 1.5 miles closer to the source of nectar, returned to their old location as returned to station F-3. Field bees in much reduced numbers continued to return to their old locations on the second day after the colonies had been moved. These were again collected and taken to the new locations. The third day was a poor day for flight, and only a few hundred bees were found flying around the old location of station F-3. As in the former instances, the returned bees were laden with pollen, much of which was from sweetclover, and with nectar.

The fact that so many bees returned to their former locations was, without doubt, due to the similarity of the entire territory and further to the fact that the bees that returned had evidently worked in the same locations within the irrigated area as they had before being moved. It is possible that other bees may have been lost on the bad-lands.

The average daily gain of the colonies that were not moved showed practically the same relation to distance as during the previous period (table 9). The average gain of the colonies 0.5 mile from the nectar-producing area was again greater than that of the colonies located within it (table 10).

TABLE 9.—Average daily net gains in weight of colonies originally located at the stations where consolidations were made, during the 3 experimental periods in 1929

Station	Average daily net gain, in kilograms, during—			Station	Average daily net gain, in kilograms, during—		
	July 3 to 15	July 17 to 29	July 31 to Aug 3		July 3 to 15	July 17 to 29	July 31 to Aug 3
A-0	0 884	1 011	1 006	E-2	0 923	1 154	
B-0.5	1 020	1 718	1 507	F-3	.735	.690	
D-1.5	.952	1 261	1 148				

TABLE 10.—Daily average net gain in weight of the colonies at the different stations during the third period, July 31 to Aug. 9, 1929

Station	Colo- nies	Average gain per colony, in kilograms, on--							Total of daily average gains in weight	Average daily gain in weight for 10-day period
		July 31	August							
			2	3	5	6	8	9		
	Number								Kilograms	Kilograms
A-0	13	0 30	2 93	1 71	2 07	1 36	2 17	0 90	11.44	1.144
B-0.5	14	.47	2 97	1.98	2 57	1.57	2 61	.98	13.15	1.315
D-1.5	13	.43	3.47	1 81	1.96	1 05	1 69	.69	11 10	1.110

Table 8 shows that in a majority of cases colonies located at the more distant stations during the second period of 1929 made greater gains in weight when moved nearer the source of nectar. In many instances the average daily gain of such colonies was greater than was that of the colonies at the stations at which the colonies had been consolidated (table 9). In each case the net gain in weight of the colonies was greater at station C-1 than it was after they were moved

within the producing area. This seemed important, as it agreed with the average results obtained in the previous periods.

DISCUSSION OF EXPERIMENTS IN 1927, 1928, AND 1929

The bees from all the stations carried sweetclover pollen during each of the 3 years. The bees at the most distant stations flew at least 8.5 miles before they came to the source of this pollen. Both Caucasian and Italian bees flew this maximum distance.

The average daily net gain or loss in weight of the colonies at the different stations was greater in 1928 than in 1927 or 1929 and also showed more variation between the different stations. These differences cannot be attributed entirely to distance, as some of the colonies at the more distant stations made greater gains than did colonies located at what appeared to be a more favorable distance. The difference was particularly evident between the colonies located at station H-5 and those at station G-4 and was probably due to a difference in the potential or in the actual strength of the colonies at these two stations.

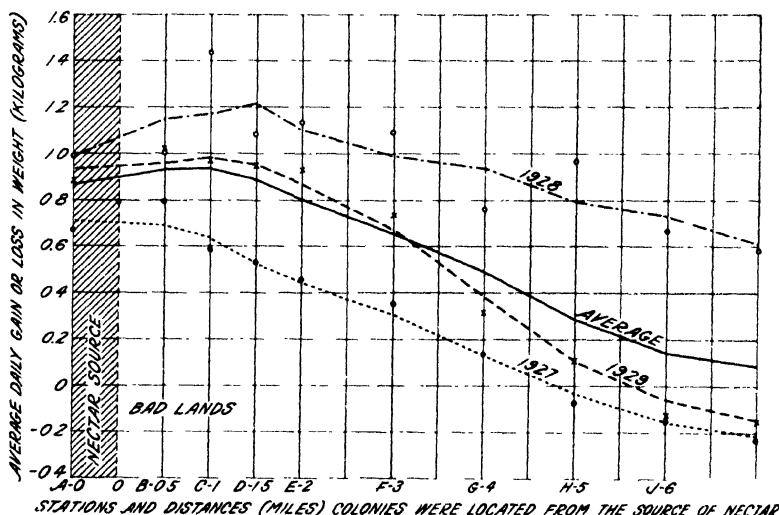


FIGURE 5.—Weight records of the colonies at stations located at known distances from a source of nectar during 1927, 1928, and 1929 in northwestern Wyoming

The same explanation does not seem to account for the difference in the average daily gain or loss in weight of the colonies within the nectar-producing area and of those located away from the source, as this difference held true during each of the 3 years. The average production of colonies located 1.5 miles from the source of nectar was slightly greater than that of colonies located within the nectar-producing area (fig. 5).

There was a gain of but 0.5 kg (1.1 pounds) in favor of the best Caucasian over the best Italian colony at each of the first 10 stations during the 3 years. This slight difference is hardly indicative of any fundamental difference between the two races in the matter of honey production under the conditions that prevailed during the experiments.

DISTRIBUTION OF HONEYBEES WITHIN A NECTAR-PRODUCING AREA

During the summer of 1930 observations were made on the distribution of bees from apiaries located within nectar-producing areas, to determine, if possible, how far they fly under such conditions and in what manner the bees distribute themselves over the available territory. The observations were made in the San Luis Valley of Colorado and on the plains west of Laramie, Wyo.

The San Luis Valley, with an average elevation of about 7,600 feet, consists of fertile irrigated farming lands devoted to the production of

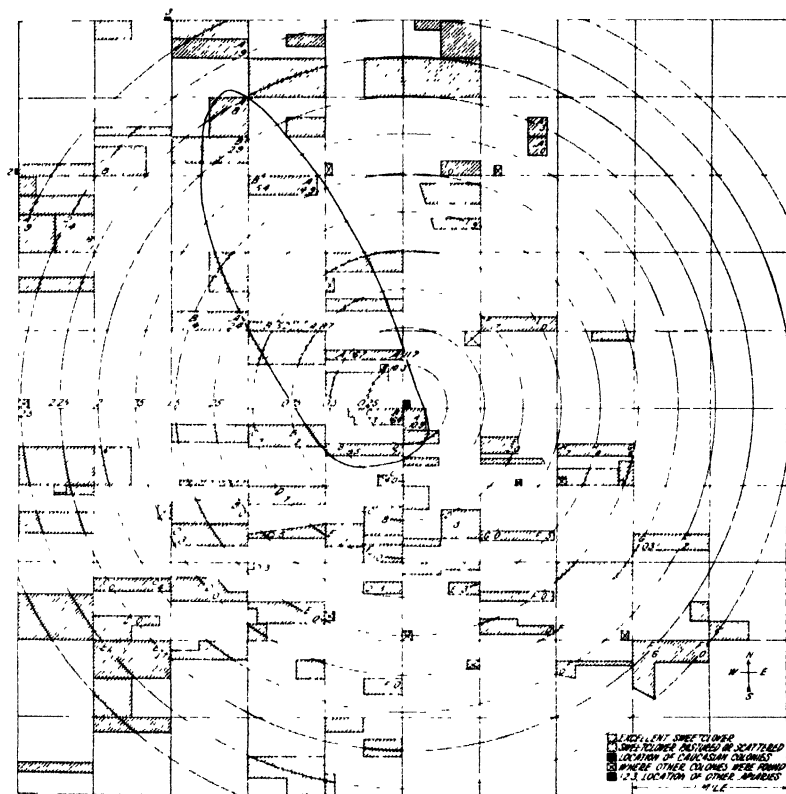


FIGURE 6.—Area in which observations were made on the distribution of bees from a central apiary in the San Luis Valley of Colorado in 1930, showing the average number of Caucasian bees counted in the different sections.

various agricultural crops and to the raising of livestock. Sweetclover is used extensively in the rotation of crops, for hay, pasture, and seed, and forms the principal source of the many carloads of honey annually produced in this valley.

In one section of the valley an area of 25 square miles containing between 2,500 and 3,000 acres of sweetclover was found to be unoccupied by commercial apiaries. Of the few stray swarms found here, all but three were of the Italian race. The location appeared to be not only an excellent place for a commercial apiary but also desirable for carrying on the observations of this experiment (fig.6).

The sweetclover in the many fields varied considerably in height, density of growth, and the stage of bloom during the period of the experiment. Probably 50 percent of the entire stand of clover remained uncut throughout the period in which the observations were being made. Many of the irrigation ditches were lined with a good growth of sweetclover. The fields of clover that had been pastured early in the spring generally came into bloom from 7 to 10 days after the fields that had not been so pastured, and for this reason an attempt was made to indicate in figure 6 the relative importance of the different fields from the standpoint of the density of stand and probable attractiveness to the bees. The fields that were pastured by sheep did not come into bloom until after the bees from the experimental apiary had oriented themselves in relation to the area as a whole.

The location west of Laramie, Wyo., where observations were continued after the close of the work in the San Luis Valley, had a

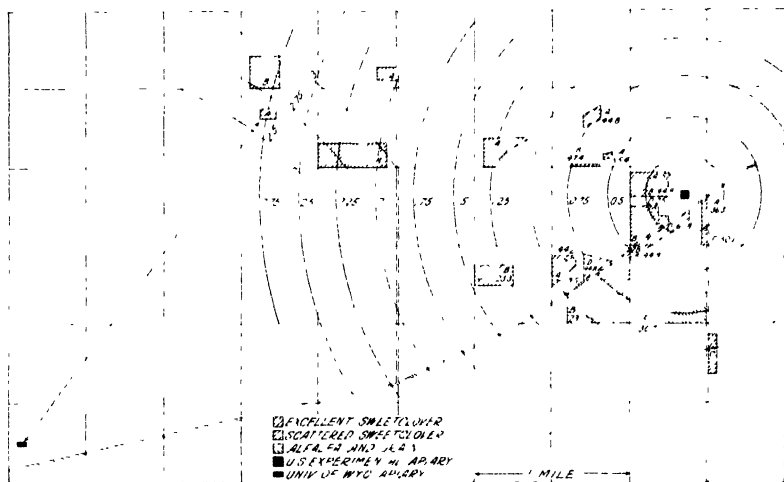


FIGURE 7.—The area in which observations were taken on the distribution of bees from the experimental apiary of the intermountain bee culture field laboratory, near Laramie, Wyo., in 1930, together with the average number of bees counted in different portions of the area. Y at the 1.5-mile point indicates a station at which observations were made, but at which no bees were found.

much more limited range of pasturage, as can be seen readily by comparing figures 6 and 7. The greater portion of this territory, however, was visited almost exclusively by bees from the experimental apiary, consisting of 42 colonies, and from a smaller apiary of 11 colonies situated about 4.5 miles away. The bees from these two apiaries, which were about 2.5 miles apart, worked to a slight extent in the intervening fields of sweetclover.

Since all the stray swarms found within the area in the San Luis Valley and within flying distance of it were Italian bees, with the exception of the three colonies of Caucasian bees mentioned, which were disposed of, it was decided to establish an apiary of Caucasian bees in the center and to use the natural color of the bees as an aid in making the observations. Accordingly, an apiary of 58 colonies, in which most of the bees were well-marked Caucasians, was established in the central position indicated in figure 6. All colonies were manipu-

lated as little as possible and were provided with ample storage space at all times.

To determine the direction of the flight of bees from an apiary, one or more observers walked around it at a distance great enough that the sound made by the bees in the apiary could not be heard. By walking slowly, stopping every few feet to listen for the sound of flying bees and to watch the direction of their flight, it was possible not only to determine the directions in which the bees flew, but also to estimate the relative number flying in any direction.

To determine the distribution of the bees over any given area, three observers took counts of bees as they walked slowly through previously designated fields of sweetclover for a given period of time. Through practice gained by the observers working together before the observations were started and several times during the experiment, it was found that, with the aid of hand tallies, each observer could count the bees in an area approximately 6 feet wide by 60 paces long during a 5-minute period. The observers were assigned to different sectors so that each covered the entire area under observation at one time or another. This procedure tended to equalize the personal equation. The observers took records at the same distances from the apiary and also at the same time, assuring thereby that the observations made at the various distances from an apiary were under identical conditions.

Bees were also marked in the fields with distinctive colors for later identification.

No records were made on days when the wind was blowing, as it was found that bees could not be counted accurately when the sweetclover plants were swaying to any great extent. As the afternoons were generally windy, the counts were for the most part made in the forenoons.

OBSERVATIONS IN THE SAN LUIS VALLEY

The observations taken in the San Luis Valley brought to light much information not anticipated before the work was started. The bees from the experimental apiary oriented themselves the morning after they were established, but no observations were recorded for several days. Most of the sweetclover fields were in good bloom at the time the bees were placed on location. It was soon evident, however, that in spite of the fact that good fields of sweetclover were available on at least three sides of the apiary, with the best fields situated in a southwesterly direction, the bees flew quite generally in a northwesterly lane of flight.⁵ A second lane of flight was in a southwesterly direction, while comparatively few bees flew in the other direction.

The data in table 11 and figure 6 indicate that the greatest concentration of bees occurred in the fields adjoining the apiary to the west and northwest and was pronounced for a distance of 0.25 to 0.5 mile. The concentration of bees seemed to be greater in field B, which was adjacent to the apiary on the southwest, than in field A, also adjacent but southeast of the apiary. Only a comparatively few bees were ever found working in the fields southeast of the apiary. The greatest

⁵ The term "lane of flight" denotes the direction and manner in which bees fly from an apiary. It is used by many beekeepers, especially in the commercial beekeeping area of the Rocky Mountain region, in a broader sense than the term "line of flight" implies. The latter term is used most frequently to denote the direction and flight of individual bees, or of a swarm, whereas the former denotes the flight of many thousands of bees flying more or less independently but in the same general direction over a narrow angle from the apiary. The two terms are used somewhat synonymously.

concentration of bees was found 0.25 mile west of the apiary, where on an average 17 bees were counted during each 5-minute period. The next greatest concentration occurred in the field 0.5 mile northwest of the apiary (A-0.5). Bees were found in greater numbers in each of five fields northwest of the apiary than in any other fields. The bees were also found to have flown as far as 2.75 miles northwest from the apiary, 2.5 miles southwest, and 2.25 miles to the southeast. The fact that there were at least 1,100 acres of sweetclover in the southwestern quarter of the area, 930 acres in the northwestern quarter, only 220 acres in the northeastern quarter, and approximately 430 acres in the southeastern quarter had little effect on the density of the distribution of the bees.

TABLE 11.—Distribution of bees in the San Luis Valley of Colorado, in the area shown in figure 6

Quarter	Station	Counts	Bees	Average bees per count	Quarter	Station	Counts	Bees	Average bees per count
		Number	Number	Number			Number	Number	Number
Northeastern.	F-0.75...	7	12	1.7	Northwestern.	B-2...	12	35	2.9
	I-1...	9	0	.0		B-2.25...	9	10	1.1
	I-1.25...	8	7	.9		A-2.25...	11	89	8.1
	I-1.5...	9	5	.6		A-2.5...	7	13	1.9
	A-1.75...	6	0	.0		B-2.5...	1	5	5.0
	A-2...	6	2	.3		C-2.5...	8	6	.8
	A-0...	26	284	10.9		D-2.5...	15	21	1.4
	E-0.75...	4	8	2.0		A-2.75...	13	12	.9
	D-0.75...	8	10	1.3		B-0...	16	362	16.4
	G-1...	3	0	.0		C-0.25...	23	399	17.3
Southeastern.	H-1...	9	4	.4	Southwestern.	D-0.25...	23	164	7.1
	E-1.25...	4	1	.3		B-0.5...	22	209	9.5
	F-1.25...	2	1	.5		C-0.5...	8	32	4.0
	G-1.25...	2	0	.0		B-0.75...	17	36	2.1
	H-1.25...	6	5	.8		C-0.75...	8	14	1.8
	F-1.5...	3	0	.0		C-1...	10	7	.7
	G-1.5...	7	4	.6		D-1...	6	4	.7
	F-1.75...	4	0	.0		E-1...	6	7	1.2
	G-1.75...	26	1	<.1		F-1...	4	4	1.0
	E-2...	4	0	.0		B-1.25...	3	0	.0
Northwestern.	F-2...	6	1	.2		C-1.25...	2	1	.5
	F-2.25...	8	5	.6		D-1.25...	4	1	.3
	F-2.5...	9	0	.0		D-1.5...	12	3	.3
	A-0.25...	23	270	11.7		E-1.5...	4	4	1.0
	B-0.25...	4	41	10.3		C-1.75...	3	1	.3
	A-0.5...	29	483	16.7		D-1.75...	5	0	.0
	A-0.75...	23	200	8.7		E-1.75...	4	0	.0
	B-1...	25	129	5.2		C-2...	8	3	.4
	A-1.25...	5	12	2.4		C-2.25...	2	0	.0
	A-1.5...	33	161	4.9		D-2.25...	4	0	.0
	B-1.5...	5	2	.4		E-2.25...	6	1	1.7
	B-1.75...	33	178	5.4		E-2.5...	8	1	.1

The data in table 11 and figure 6 show that beyond a 0.5-mile radius the bees distributed themselves principally in one direction.

The hives in the experimental apiary were located in the southwestern corner of a sparsely wooded pasture and faced south. Adjacent to the apiary at the southwest stood a small group of trees which seemingly did not interfere with the flight of bees. The general direction of the prevailing wind was from the southwest, although at different times it shifted to all other directions. Italian bees were found in all fields in which observations were made, but apparently never in sufficient numbers to be a factor in excluding other bees. No explanation in regard to landmarks, character of the fields, or climatic factors could be found to account for the way in which the

bees distributed themselves in relation to the area as a whole, although the fact that the fields of sweetclover which had been pastured, such as those in which stations B-1.5 and A-1.25 were located, did not come into bloom until after the bees had become oriented undoubtedly was responsible for the low counts.

In another portion of the San Luis Valley a number of apiaries were located in fairly close proximity to one another (fig. 8). As the bees from the experimental apiary mentioned above had not distributed themselves in all directions over the available territory, it

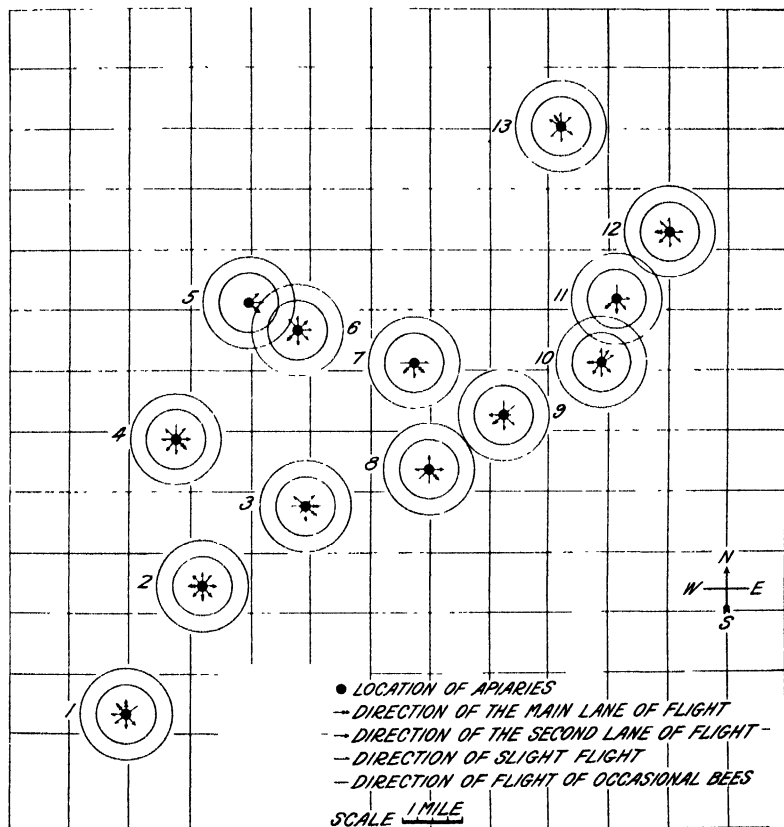


FIGURE 8 Relative location of 13 apiaries in the San Luis Valley of Colorado, showing the directions in which the bees flew from each apiary.

was thought that a determination of the directions in which the bees flew from the apiaries in another part of the same valley might give some additional information on the distribution of bees when located within a good nectar-producing territory.

It was found that the bees from 9 of the 13 apiaries had only one major lane of flight, and that of the remaining 4 the bees from only 2, nos. 2 and 7, were flying in major lanes of flight that were in different directions. Bees from only two apiaries, nos. 11 and 5, were trespassing within a radius of 1.5 miles in the territory of the major lane of flight of apiaries 10 and 6. In the case of apiary 5 the main

flight went directly over and slightly to one side of apiary 6, so that the bees from apiary 5 were actually working in a territory south-east of apiary 6. The bees from apiary 9 were apparently concentrating their efforts in an area lying between them and that of apiary 8, but as the bees from the latter were flying mainly in another direction the two flights did not cross. In only a few instances, and particularly in the case of apiary 2, were the bees of any apiary taking full advantage of their location, as judged by the directions in which they were working in relation to the fields of sweetclover that surrounded them.

The direction of the prevailing winds appeared to have no definite relation to the principal directions in which the bees were flying from the different apiaries. As the directions of flight evidently depended on a major source of supply to which the bees had oriented themselves at the time the plants came into bloom, it is only reasonable to suppose that the direction of flight would depend on a continuance of the nectar flow in that direction. If for any reason the source of food was reduced or if a better source of nectar was discovered in another direction, the direction of flight might be changed accordingly.

Such a contingency was investigated by the writer during the summer of 1929. For some unexplained reason, at certain times nearly every summer bees from a commercial apiary near Worland, Wyo., were in the habit of leaving a fertile valley in which they were located to fly over a stretch of badlands to a much smaller valley and bring back nectar in quantity and quality similar to what they had been gathering. The distance across the badlands was 4.6 miles. On other occasions, however, the bees had been observed to fly in a slightly different direction, making the distance covered even greater. In order to leave the valley in which they were located, the bees had to fly over a steep rise of land that was estimated to be at least 150 feet higher than the apiary. The badlands were extremely rough, there was no source of water or nectar between the two valleys, and the flight was not in the direction of the prevailing winds.

OBSERVATIONS NEAR LARAMIE, WYO.

As stated previously, the nectar-producing flora in the vicinity of the apiary west of Laramie, Wyo., belonging to the intermountain bee culture field laboratory, was much more limited than that in the San Luis Valley of Colorado; consequently, the observations were confined to a smaller area (fig. 8). The distribution of the bees was found to be somewhat uniform within a radius of 0.75 mile, but beyond this distance the number of bees found in any field decreased considerably, as did also the available sources of nectar. Field A-1.25 and the greater portion of B-1.5 were cut for hay soon after the observations were started. Field B-2 was in good bloom but consisted of very short clover that had not been irrigated and, although it was examined on several occasions, no bees were ever found there. Only a few bees were found in field A-2.75 and in B-2.75, but most of these were shown to have come from the apiary of the University of Wyoming, 2.5 to 2.75 miles to the southwest.

As the colonies in this location were stronger than those in the experimental apiary in the San Luis Valley, the results of the two sets

of observations cannot be compared directly. Possibly this factor, plus the restricted territory of the Laramie location, was responsible for the noticeable increase in the numbers of bees found working in the areas under observation.

DISTRIBUTION OF MARKED BEES

A number of bees were marked distinctive colors in each of the 3 fields in the San Luis Valley and in 3 fields west of Laramie, in an attempt to identify the colonies from which they came and thus to establish the distances they flew, and also to learn whether the same bees return to the same fields on successive days.

In field D-1.5 in the San Luis Valley 150 bees were marked with a green dot. On four successive days thereafter green-marked bees were found in the same field. A beekeeper told the writer that he had found 2 green-marked bees in his apiary 2.5 miles southeast of this field 2 days after the bees were marked. No green-marked bees were ever found in the experimental apiary.

In one corner of a much larger field, B-1.75, 190 bees were marked another color. Over a period of 12 days thereafter some of these marked bees were found working in the same small corner of the field in which they were marked. During the same period 12 bees with the same markings were found in the experimental apiary, and 1 was found working in field A 7 days after the first bees were marked.

In a third field, A-2.25, 10 bees were marked still another color and, although none of these were ever found again in the same field, 4 of them were seen in the hives of the experimental apiary on the third, sixth, and eighth days after they were marked.

In an effort to determine from which apiary the bees came that were found working in field A-2.5 (fig. 7) near Laramie, 126 Caucasian and 42 Italian bees were marked in that field. Two days later, when only a few bees were working, 3 marked bees were seen in the same field. On the following day 1 of the marked bees was found in the apiary connected with the intermountain bee culture field laboratory and 47 in the apiary belonging to the University of Wyoming. These 47 bees were scattered in 10 of the 11 colonies in this apiary, although in 1 colony 25 marked bees were counted.

In another small field located beyond the flying range of these 2 apiaries but within 2.5 miles of 5 other apiaries, which ranged from 6 to 37 colonies in size, 827 bees were marked. During a 30-minute examination of this field on each of 2 successive days thereafter, 36 marked bees were observed out of a total of 493 bees counted. The colonies in the 5 apiaries were then searched, with the result that marked bees were found in each apiary, and a total of 127 marked bees were located (table 12). Although 7 colonies of bees were found in the field in which these bees were marked, only 22 marked bees were recovered in these colonies, in contrast to 31 in the same number of colonies 0.5 mile west of the field. In another apiary 2.25 to 2.5 miles distant, from which the bees had to fly over the city of Laramie, Wyo., as well as over a mile or more of other territory, in order to reach the small field of clover, 5 marked bees were recovered.

TABLE 12. —*The distribution of bees from 5 apiaries in relation to a small field in which 827 bees were marked*

Distance of apiaries from field (miles)	Colonies in apiary	Colonies examined	Colonies with marked bees	Bees found in each colony (number)	Total marked bees found
	<i>Number</i>	<i>Number</i>	<i>Number</i>		<i>Number</i>
Same field	7	7	7	6, 4, 2, 2, 3, 2, 3	22
0.5..	7	7	7	1, 6, 2, 6, 4, 6, 6	31
0.75..	43	37	11	1, 1, 1, 1, 1, 3, 1, 1, 1, 2, 1....	14
1.25 to 1.5..	29	23	9	1, 12, 3, 1, 28, 1, 4, 3, 2	55
2.25 to 2.5..	7	6	3	1, 2, 2..	5

SUMMARY

Estimates of various writers have placed the flight range of honeybees at distances of from 1 to 7 or more miles, but little experimental evidence has been presented. It is also generally believed by beekeepers that bees will fly in all directions from an apiary in quest of nectar and pollen. Regulations governing the location of apiaries in respect to the control of bee diseases, from the viewpoint of honey production, and to prevent bees from becoming nuisances, vary widely in their requirements owing to differences in the assumed distances that bees can fly.

When colonies were located on a prairie in Wyoming, during a dearth of nectar, it was found that the bees would fly but 1.5 miles for artificial stores and at least 3 miles for pollen and nectar.

When bees were separated from a given nectar-producing area by the badlands, with no other source of food intervening, they flew a maximum distance of at least 8.5 miles. Colonies located within 0.5 to 2 miles of a given source of nectar made gains in weight, over a period of 3 years, as great as, or greater than, similar colonies located within the same nectar-producing area, and colonies lost in weight when placed 5 miles or more from nectar.

When colonies were moved for varying distances up to 3.5 miles, many bees went to the fields and then returned to their former locations. The return of such bees was undoubtedly influenced by the fact that the colonies had been moved into surroundings that were similar to their former locations and also by the fact that the bees worked in the same territory in which they had worked before being moved.

Experiments on the distribution of bees from apiaries located within a nectar-producing area showed that bees have a tendency to fly in only one or two major lanes of flight, neglecting similar forage plants in other directions. Bees would fly for 2.75 to 4.6 miles in one direction when located within a nectar-producing area and confine their efforts to working in that direction rather than in nearer fields of seemingly equal attractiveness. In one series of observations, bees were found to work most numerous in fields located within 0.5 mile of their apiary. Where several apiaries were located within the same general territory, the major lanes of flight from the different apiaries did not indicate so much trespassing of the bees from any one apiary on the territory of another as might be expected from the proximity of their locations. Where the nectar supply was limited, bees from

several colonies in each of five apiaries worked in the same small field, some of the bees flying at least 2.5 miles to reach the field in question.

The results confirmed the conclusions of previous investigators that bees have a tendency to return to the same portion of a field, or to the same small field, on successive days for nectar and pollen, even though other areas of the same forage plant are nearer.

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METHODS OF CONTROLLING POLLINATION IN THE PECAN¹

By HAMILTON P. TRAUB, *horticulturist*, and L. D. ROMBERG, *agent*, *Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

A workable method for the control of pollination in the pecan is a prerequisite to further progress in the study of pollination problems and in the prosecution of practical breeding work. Various methods have been employed by different workers in an attempt to control pollination in the pecan. Risien (3, 4)² first used large paper bags for covering the pistillate flowers. The bags were tied on the twig below the new growth. Later he used small gelatin capsules, pushing them over the pistillate clusters and stuffing the open end with cotton. This method was not satisfactory because the capsules softened when wet. He also used small electric light bulbs which were fastened to a support to lessen the weight on the shoot. Stuckey (5) used closely woven lawn cloth bags; Adriance (1) used glassine bags; and Reed (2) used "medicated" cotton covers, a method previously employed by Van Fleet.

In 1930 Woodroof (?) pointed out that apparently all the work done up to that time on the control of pollination in the pecan was open to question because of the failure to prove definitely that foreign pollen had been excluded. The experiments herein reported were therefore undertaken to devise, if possible, a more effective method for controlling pollination in the pecan.

MATERIAL AND METHODS

The trees used in the experiments were located in the Pearce pecan orchard on the Colorado River near Austin in central Texas and in the Kemper pecan orchard on the Red River near Denison in north-eastern Texas³ (fig. 1).

The method finally adopted for excluding pollen was to cover the pistillate terminal with a finely woven cloth bag dipped in boiling paraffin of low melting point. In such a bag the qualities of pliability, durability against winds and rain, transparency to light, relatively light weight, and capacity to exclude wind-carried pollen effectively were apparently obtained in sufficient measure. This fundamental method was used in several variations and was checked against the commonly used cotton-cover method. The types of covers tested were as follows:

(1) Large nonventilated paraffined cloth bags (fig. 2, A).—A commercial mailing sack, 6¾ by 13½ inches, was pulled over the shoots of the twig, and the neck of the bag was securely tied to the twig. The string was drawn together at two or three places over a space of about 1 inch to reduce the possibility of pollen entering at that point.

¹ Received for publication Mar. 13 1933, issued September 1933.

² Reference is made by number (italic) to Literature Cited, p. 296.

³ The experiments at Denison, Tex., were carried on cooperatively with the Texas State Department of Agriculture.

(2) Large ventilated paraffined cloth bags (fig. 2, B).—Bags similar to those just described were used, but ventilation was provided. This was accomplished in two ways. In one set of tests a corner of each bag was cut out and filled with a cotton plug and the neck of the bag was tied down on a cotton layer about $1\frac{1}{2}$ to 2 inches in diameter. In the other tests ventilation was provided only by means of a cotton layer in the neck of the bag.

(3) Small nonventilated paraffined cloth bags.⁴—These bags measured $1\frac{1}{2}$ by $3\frac{3}{4}$ inches when flattened out, and when opened were approximately 1 inch in diameter. Some were attached by tying the neck with a string only; others by tying in the same manner but thereafter sealing the neck with melted paraffin.

(4) Small ventilated paraffined cloth bags (fig. 2, C and D).—These bags were identical with those just described, but when they were attached the neck of each was stuffed with cotton, which not only provided ventilation but also acted as padding for the tender shoot. One leaf was usually pinched off to make room for the cotton padding, and 2 to 5 of the small young leaves immediately below the blossoms were placed in the bag. A pad of cotton was wrapped around the shoot at the selected point, and the open bag was pushed over the pistillate cluster, terminal leaves, and cotton. The bags were put on as far out on the



FIGURE 1.—Typical pecan trees used in the experiments: A, Tree 25 feet high in Kemper orchard, Denison, Tex.; most of work done from the ground; B, Tree 40 feet high in Pearce orchard, Austin, Tex.; note 22-foot ladder necessary to carry on work.

tender shoots as possible without tilting the shoot downward too much. The tendency to droop was overcome as the shoots grew stronger. Two methods were used in fastening the bags. Half of the bags for each treatment were lightly stuffed in the neck with cotton and were secured by a string tied around the neck (fig. 2, D). The other half were fastened by using cotton stuffed tightly enough into the neck of the bag to hold it in place without tying (fig. 2, C).

(5) Cotton covering.—From large bundles of commercial quilt cotton whole layers about 1 inch thick (loose) were taken off. Pieces 3 to 4 inches square were torn from these layers and made into covers by rounding and thinning the edges and pushing the sides together over the fingers so as to form a cup-shaped or bag-shaped mass. The cover was pushed over the terminals and tied below the blossoms and outermost leaves with a string. The cotton surrounding the blossoms was one half to three fourths of an inch thick, forming a ball $1\frac{1}{2}$ to 2 inches in diameter on the shoot (fig 2, E). Usually it was necessary to remove a leaf from the shoot to facilitate the tying of the cotton in place.

Two methods of applying pollen, designed to exclude foreign pollen, were employed in the experiments, viz, (1) the use of a hypodermic

⁴ The value of cellophane bags for this purpose is being tested.

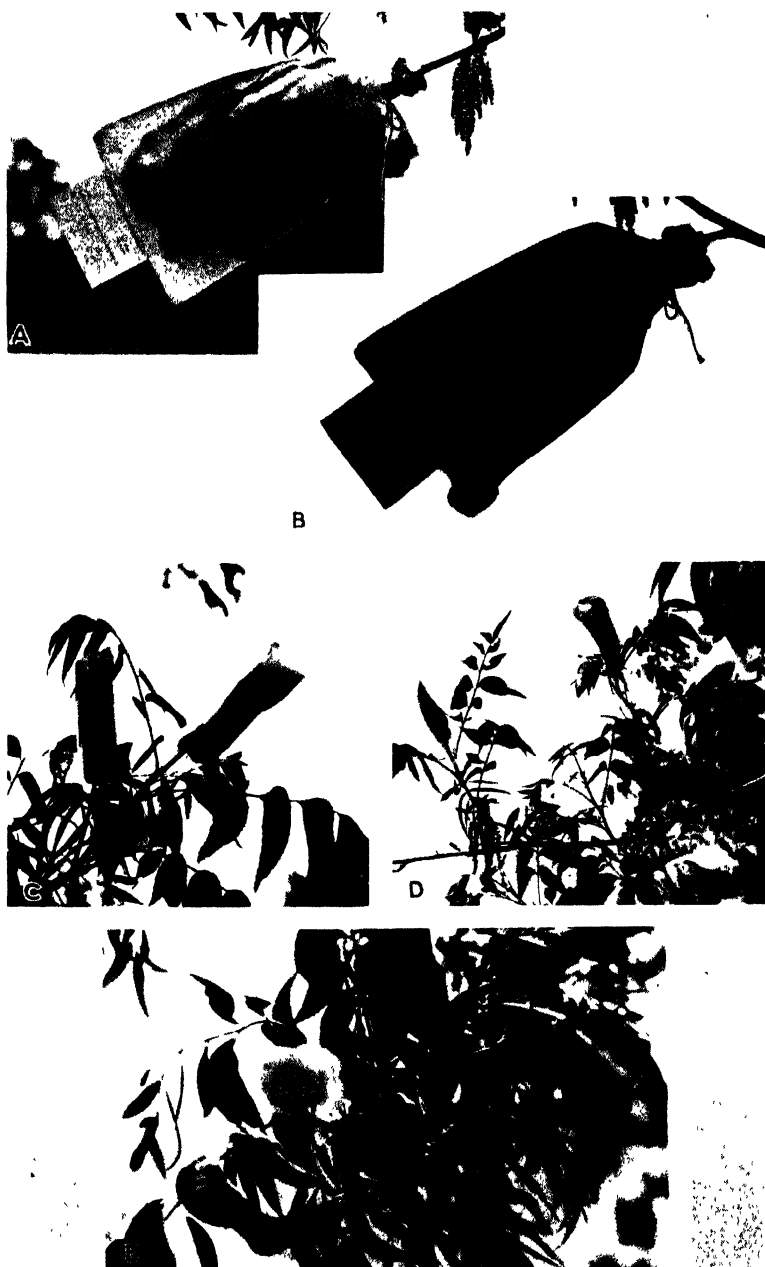


FIGURE 2.—Types of covers used in pollination tests: A, Large nonventilated paraffined cloth bag; B, large ventilated bag; C, small ventilated bags, not tied, D, small ventilated bags, tied, E, cotton cover.

syringe (fig. 3) which made it unnecessary to remove the bag, and (2) the application of the pollen after removal of the bag, at an hour of the day when there was little possibility of pollen being carried in the air, and immediate replacement of the bag. When the syringe was used, the needle was thrust through the paraffin-impregnated cloth and the pollen injected into the bag. Immediately after the needle was withdrawn the punctures were closed by smearing with paraffin of low melting point. In the case of the small ventilated bags the needle was inserted either through the cotton pad or as previously indicated.

It has been shown that no pecan pollen is shed when the relative humidity of the air is 85 percent or higher (7). Actual tests with petrolatum-coated glass slides supported on a rack after the method used by Waugh (6) and Woodroof (7) indicated that there was little or no pollen in the air between the hours of 5 and 7 a.m. It appeared safe, therefore, to carry on the operations between these hours. In certain tests, bags were removed after 7 a.m. to determine the probability of contamination and whether pollination later in the day would affect the set.

In order to get a controlled cross it is necessary to procure uncontaminated pollen. A promising method consists in picking the catkins before any pollen has been shed, washing them repeatedly in water between the hours of 5 and 7 a.m., and drying them in a desiccator after the excess water has been removed with a sterile towel. After the pollen is shed in the desiccator it is run through a fine-mesh sieve to remove larger particles. The pollen is then stored in glass vials stoppered with cotton and covered with a vial of larger size. The quantity desired in the pollen container of the syringe is sucked through the hypodermic needle after the needle has been thrust through the cotton plug. When the variety of pollen is to be changed both the sieve and the entire syringe

are sterilized by the use of 95 percent ethyl alcohol. This method was not followed throughout in the preliminary work, since the question of making a particular cross was not involved.

In part of the work the hypodermic syringe employed was the type ordinarily used by physicians, but this was not entirely satisfactory for the purpose. It was therefore modified by putting on a large rubber bulb such as is used on syringes to fill automobile batteries. This bulb, which served as a container for the pollen, held a sufficient quantity for a large number of pollinations. Other improvements in the syringe which will allow the insertion of the needle at any angle and also provide a constant quantity of pollen, are now under consideration.

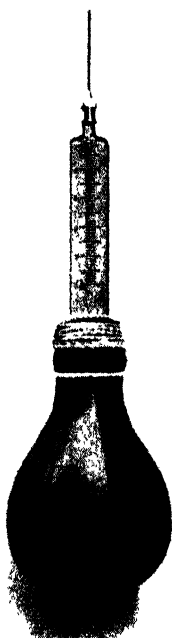


FIGURE 3—Syringe used in pollinating

The various treatments consisted in utilizing the types of covers described and pollinating by needle injection; also, by removal of the covers at an hour when there was little or no pollen in the air and subsequent replacement of the covers. Check treatments included covers that were not removed for the bagging period and covers that were removed for a time equivalent to that required for pollination and then replaced. In two instances the covers were removed and pollen applied after 7 a.m., with replacement. At the time when these treatments were given, some covers were removed permanently.

Records of the number of clusters and the number of nuts per cluster were taken (1) when the bags were finally removed, (2) in early July, (3) in August, and (4) at the time of harvesting. When the July check was made, bags of mosquito netting were placed over the clusters to protect them from further damage by casebearers and to catch any nuts that might drop. At this time it appeared that all nuts that might drop owing to lack of fertilization were off. Later much damage to the nuts at the Red River orchard was caused by a severe storm and by scab. Conclusions as to results of the different methods must therefore be based chiefly upon the July data.

PRESENTATION OF DATA

The results of the experiments are presented in tables 1 and 2.

TABLE 1.—Results of experiments with different methods for controlling pollination in the pecan in Texas in 1931

KEMPER ORCHARD AT DENISON

Variety, tree, and kind of bag or cover	Bags of covers originally placed	Bags or covers usable	Bags or covers per treatment	Nature of treatment	Date bags or covers were originally placed	Date treatment given	Date bags or covers were permanently removed	Nuts in usable bags when bags or covers were removed			Nuts on tree July 1			Data on nuts Aug 18			Nuts harvested Oct. 22			
								Nuts when bags or covers were removed			Nuts when bags or covers were removed			Destroyed by storm				Dropped		
								No.	No.	Per cent	No.	No.	Per cent	No.	No.	Per cent		No.	No.	Per cent
Halbert (tree no. 96).																				
Large nonventilated bag.	121	119	21	Check, not pollinated, neck not paraffined.	May 3.		May 31.	48	29	74	0	0	0	0	0	0	0			
			20	Check; not pollinated, neck paraffined.	do.		do.	39	19	45	0	0	0	0	0	0	0			
			20	Pollinated by needle injection.	do.	May 16.	do.	49	21	64	0	0	0	0	0	0	0			
			20	Removed, pollinated, replaced, before 7 a m.	do.	May 17.	do.	44	28	67	4	6	0	0	0	4	3			
			20	Removed, not pollinated, replaced, before 7 a m.	do.	do.	do.	46	19	44	0	0	0	0	0	0	0			
Halbert (trees nos. 265, 211, and 246).			18	Bags removed and left off.	do.		May 18.	38	14	33	14	42	2	0	0	13	13			
Large ventilated bag.	60	58	20	Check, not pollinated.	May 6, 7.		June 1.	52	44	136	0	0	0	0	0	0	6			
			20	Pollinated by needle injection.	do.	May 16.	do.	57	39	112	13	11	7	0	0	6	6			
			19	Removed, pollinated, replaced, before 7 a m.	do.	May 17.	do.	59	54	184	20	11	13	2	3	2	2			
Halbert (trees nos. 211 and 179)			10	Check, not pollinated.	May 4.		do.	10	9	30	0	0	0	0	0	0	0			
Small ventilated bag, tied.	40	40	11	Pollinated by needle injection.	do.	May 16.	do.	11	11	32	25	78	0	0	0	25	23			
			10	Removed, pollinated, replaced after 7 a m.	do.	May 17.	do.	10	9	27	18	66	0	0	0	16	16			
			9	Removed, not pollinated, replaced after 7 a m.	do.	do.	do.	9	9	24	2	8	0	0	0	2	2			
Small ventilated bag, not tied.	40	40	11	Check; not pollinated.	do.		do.	11	11	40	0	0	0	0	0	0	0			
			9	Pollinated by needle injection.	do.	May 16.	do.	9	30	15	50	2	3	0	0	9	9			
			10	Removed, pollinated, replaced, after 7 a m.	do.	May 17.	do.	10	2	23	16	70	15	0	0	1	1			
Summary of small bags both tied and not tied.	80	80	10	Removed; not pollinated, replaced, after 7 a m.	do.	do.	do.	10	9	36	1	3	0	0	1	0	0			
			21	Check, not pollinated.	do.		do.	21	490	70	0	0	0	0	0	0	0			
			20	Pollinated by needle injection.	do.	May 16.	do.	20	419	62	40	64	2	3	0	34	32			
			20	Removed, pollinated, replaced, after 7 a m.	do.	May 17.	do.	20	416	50	34	68	15	0	0	17	17			
Halbert (trees nos. 324 and 265).			19	Removed, not pollinated, replaced, after 7 a m.	do.	do.	do.	19	418	60	3	5	0	0	0	3	2			
Cotton.	60	49	14	Check, not pollinated.	do.		May 31.	14	11	47	26	55	1	0	0	25	21			
			18	Removed, pollinated; re-covered.	do.	May 18.	do.	18	18	61	53	87	0	0	1	52	52			
			17	Removed, pollinated, left uncovered.	do.	do.	do.	17	16	58	50	86	0	0	1	49	48			

TABLE 2.—*Loss of nut clusters from covered shoots*

Type of cover	Halbert variety (Kemper orchard) ^a			Success- variety (Pearce orchard), abscission during 30 days after removal ^a
	Abscission while bagged	Abscission during 30 days after removal	Total	
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Large nonventilated bag ---	36	57	93	81
Large ventilated bag ---	7	76	83	89
Small nonventilated bag (not paraffin-sealed) ---				60
Small ventilated bag ---	0	6	6	
Cotton ---	0	0	0	75

^a The percentage of abscission for the Pearce orchard is based on the number of clusters on the tree at cover removal, for the Kemper orchard it is based on the number when first covered. The data are only for those clusters that were in the group to be pollinated by removal of covers.

In a consideration of the effect of the bags on the shoots, the length of the time of bagging and the effect of the size and ventilation of the bags are important. Apparently a valid criterion for pistil receptivity in the pecan has not yet been demonstrated (7, 5, 1). For this reason it was difficult to determine how long the bags should remain on, and they may possibly have been left on longer than was actually necessary. As would be expected, growth was stimulated in the shoots bagged during the early part of the season (from the middle to the end of April), which was relatively cool. However, as the warmer weather of May came on, there seemed to be a reversal of this process, and a retarding effect was noticed. In all bags a shedding of foliage was observed, due apparently to the changed environmental conditions caused by the cover. A tendency of the young nuts to abscise seemed to be positively correlated with injury to the foliage. After the bags were removed the shedding of foliage ceased, but the young nuts continued to abscise abnormally in proportion to the abscission exhibited during the bagging period. In the case of the large bags placed on the Success variety at the Pearce orchard, approximately 10 percent of the bagged shoots produced staminate flowers from lateral buds which would not normally have been produced until a year later. In several cases pistillate flowers were borne on these staminate inflorescences intermingled between the staminate florets. Cotton covers gave varying results at the two orchards, possibly owing to the difference in varieties or in the application of the material.

A summary of the data on the loss of clusters and nuts from shoots while covered and during 30 days after removal of covers of properly pollinated clusters is given in table 2. It will be noted that ventilation is apparently beneficial,⁵ and also that a small cover enclosing only the terminal portion of the shoot is apparently less harmful than a large one enclosing the entire shoot. Some abscission of nutlets is known to occur where clusters have not been bagged. For this reason the abscission from clusters covered with small ventilated bags does not appear to be excessive; for large bags, both ventilated and nonventilated, abscission is relatively high. It may be, however, that when a valid criterion for pistil receptivity is found, the length

⁵ Water collected in the lower end of nonventilated bags.

of time necessary to cover may be reduced to such an extent that all types of covers will be less harmful. By referring to table 1 it will be noted that the treatment of large nonventilated bags, which consisted in removing the bags permanently after 15 days, gave a 42 percent set of nuts, whereas under similar bags removed after 28 days, a set of only 6 percent occurred. In the latter case the flowers had been artificially pollinated.

In considering the degree of control secured by the various treatments, the effect of pollinating and of not pollinating, the relative effectiveness of pollinating by means of a hypodermic syringe and pollinating by removal of the cover, the relative value of the bagging methods as contrasted with the cotton method, and any indication of self-sterility or intersterility should receive attention. As shown in table 1, complete control was secured in every case where the bagging technic was employed and pollen was withheld. Similarly, nuts were set in the majority of cases where pollination was artificially attempted. Apparently there is no consistent difference in the number of sets due to the use of the syringe in pollinating and to the use of the method of removing, pollinating, and replacing before 7 a. m. (table 2). Some nuts were set on clusters similarly exposed after that hour, when pollen was known to be in the air. Partial control of fertilization was secured with cotton covers.

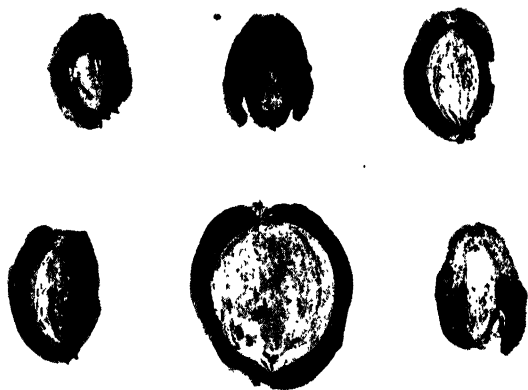


FIGURE 4. Normal and abnormal Halbert pecans from self-pollinated clusters, harvested October 22. The large nut (lower center) is normal, the nut to its left is an abnormal one from the same cluster. The others are abnormal nuts from different clusters.

Fewer nuts were set in clusters not artificially pollinated and continuously covered than in clusters that were artificially pollinated.

The relative value of the small bags, large bags, and cotton covers must be judged both by the effect of the covers on the shoots and by the degree of control secured. On this basis the small ventilated bags appear to be decidedly superior to the large bags whether ventilated or not ventilated. Cotton covers as used in this experiment allowed pollen to sift through. Birds removed some for nest building. Cotton covers are also less desirable than small ventilated bags because they do not permit pollination by needle and because much more work is required to place them on the trees, to remove them for pollination, and to replace them. With walnut work on the Pacific coast a more careful cotton-cover technic is employed, which apparently is more effective than the method used in these experiments.

Pollen from Halbert trees was used throughout this experiment. No arrested or abnormal nut development was observed except with the Halbert variety (fig. 4), which would indicate that this variety may be somewhat self-incompatible.

CONCLUSIONS

The results of one season's work carried out at two locations in Texas indicate that—

(1) The requirements of an effective covering material apparently are met by the use of a finely woven cloth impregnated with paraffin of low melting point.

(2) Controlled pollination may be effected by means of a hypodermic syringe, without removing the covers.

(3) Controlled pollination may be effected by pollinating after removing the cover and then replacing it between the hours of 5 and 7 a.m. (relative humidity 85 percent or above), under the conditions of this experiment.

(4) Cotton covers as used in the experiments gave no effective control.

(5) The use of a small paraffined cloth bag with cotton stuffed into the neck to provide ventilation and padding for the tender shoot, and pollinating by means of a hypodermic syringe give promise of excellent control easily secured, if one season's results are verified in future years. This type of cover is in every way superior to the large ventilated bag.

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BLISTER RUST DAMAGE TO NORTHERN WHITE PINE AT WATERFORD, VT.¹

By E. C. FILLER²

Senior pathologist, Division of Blister Rust Control, Bureau of Plant Industry,
United States Department of Agriculture

INTRODUCTION

White-pine blister rust, caused by the fungus *Cronartium ribicola* Diet., was first found in North America during 1905 in a nursery near Philadelphia, Pa. (6).³ Subsequent investigations have indicated that it was introduced in York County, Maine, as early as 1897, on European black currants (*Ribes nigrum* L.) imported from England (7). However, far more important introductions occurred as the result of importations of northern white pine (*Pinus strobus* L.) from European nurseries during the period from 1900 to 1910. Surveys have shown that diseased stock was planted in more than 200 locations in the Northeastern States. From these infection centers the blister rust spread to natural stands of white pine through the medium of ribes⁴ and thus became generally distributed in these States (9).

In the Northeast blister rust has seriously endangered the survival of white pines wherever they occurred closely associated with ribes, the alternate host of the fungus. White pines of all sizes are attacked and killed by the rust (10), but the time required for killing is proportionate to the size of the tree. Consequently, on many areas in the older infested regions, where the trees have not been protected by the timely eradication of ribes, the seedling pines have already been killed and the sapling growth has been severely injured, as shown by the many dead and dying trees, while among the merchantable-size trees the damage is only now beginning to accrue. Except for the presence of dying branches, the effect of the disease on the larger pines does not become readily noticeable until the girdling of the trunk causes the crown foliage above to turn yellow. In the case of large trees under eastern conditions, this sometimes requires 15 years or more. So insidious is its attack that frequently the blister rust progresses to this advanced stage before it is recognized by pine owners.

The blister rust was first found in northern Vermont in 1910. Survey records indicate that it was introduced there on northern white pine imported from Germany about 1901 and planted at Lyndonville. From this and from later plantings of infected pines imported in 1909 the disease became generally distributed in the native white pine areas of this district.

¹ Received for publication Jan. 26, 1933; issued September 1933, Cooperative investigations by the U.S. Department of Agriculture and the infested States.

² The field data on wild ribes and pines in the pasture pine type were secured in 1924 under the direction of E. H. Ford, formerly of the Division of Blister Rust Control, the data for the merchantable stand were obtained under the supervision of the writer and represent conditions existing in 1925 and 1930. The writer wishes to acknowledge his indebtedness to the staff of the Washington office, Division of Blister Rust Control, for assistance in preparing the manuscript.

³ Reference is made by number (italic) to Literature Cited, p. 313.

⁴ When not employed as a generic name, the term "ribes" is used in this paper to designate currant and gooseberry plants.

An investigation was begun in 1924 on an area at Waterford, Caledonia County, Vt., where considerable blister rust damage was beginning to occur among white pines of merchantable size. In order to prevent additional infection, initial control measures were applied in 1925. This work consisted of uprooting the ribes on and near the area. Although this procedure established control of the disease, it was of course no deterrent to the continued growth and killing action of the cankers that had originated prior to the eradication of the ribes.

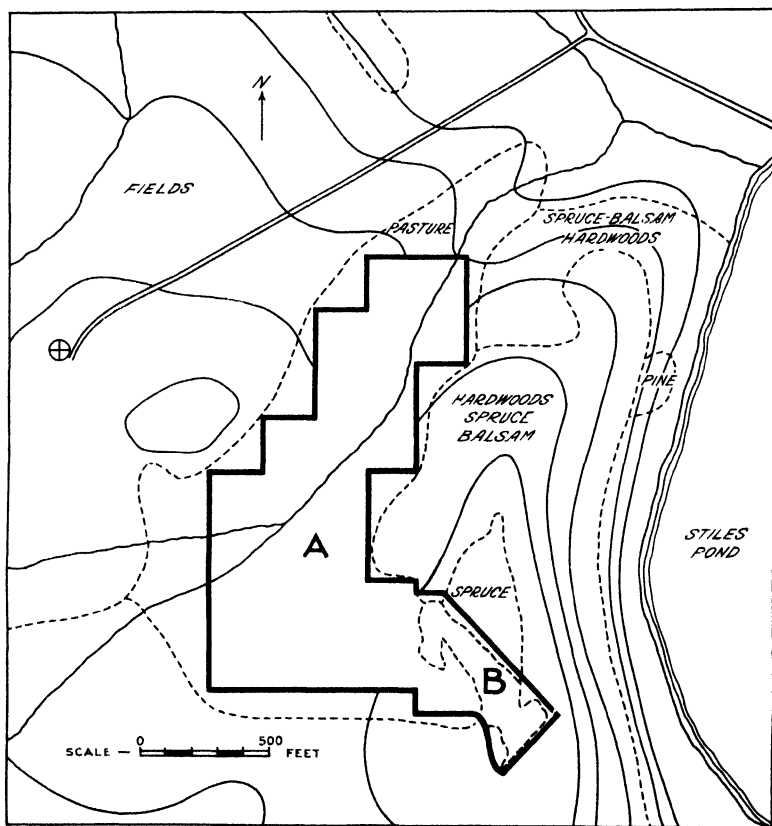


FIGURE 1. Sketch map of study area at Waterford, Vt. ⁵ Heavy black line indicates boundary of study area. A, Pasture-pine type, B, merchantable-pine type. Continuous lines (—) represent contours (40-foot intervals), wavy lines (---), streams, broken lines (---), type boundaries, double lines (==), roads. The symbol (⊕) indicates location of cultivated black currants.

Data obtained during this investigation, on the occurrence and effect of blister rust on merchantable white pine, are presented in this paper.

DESCRIPTION OF INFESTED AREA

The area studied comprises 27.43 acres located on the top and on the northeastern slope of a rolling upland plateau (fig. 1). It contains two forest types: (1) A scattered stocking of uneven-aged, bushy white pines in an old pasture of 25 acres, where in 1924 there was an

⁵ Details of types and topography were taken from a sketch map prepared by A. E. Fivaz in 1927.

average of 72 trees per acre, ranging up to 50 feet in height; and (2) a 2.43-acre mixed stand of 60-year-old white pines, uneven-aged spruce, and hardwoods. The latter unit, which hereafter will be designated as the merchantable type or stand, contained 1,740 trees, 57 percent of which were spruce, 24 percent white pine, and 19 percent hardwoods. Only 673 of these trees were over 6 inches in diameter at breast height in 1930, 52 percent being white pine, 37 percent spruce, and 11 percent hardwoods. Except for a few trees in spots where the drainage is poor, the white pines in both types are vigorous, well foliated, and rapid-growing when not infected with blister rust. As this paper is primarily concerned with blister rust damage to the white pines, discussion of the spruce and hardwoods in the merchantable type will be limited to those facts that are essential in presenting an adequate description of general forest conditions on the area.

Including 66 trees with breast-high diameters of 6 inches or less, the merchantable stand contained 416 white pines, 69 percent of which ranged from 51 to 80 feet in height and the remainder from 21 to 50 feet. Natural pruning had killed the branches on the lower two thirds of the boles. The white pines were distributed throughout the stand, the greatest concentration being on two $\frac{1}{4}$ -acre sample plots where 27 percent of the total number occurred on 21 percent of the area. The merchantable stand is bounded partly by the pasture type, but chiefly by a dense mixed growth of medium-aged conifers and hardwoods (fig. 1).

In 1924 individual plants of the wedgeleaf gooseberry (*Grossularia hirtella* (Michx., Spach) were found generally distributed in the many swampy depressions throughout the pasture type. These bushes had on the average a total of only 54 feet of live stem⁶ per acre and were partly concealed in the long grass. Three small skunk currants (*Ribes glandulosum* Grauer), totaling 15 feet of live stem, were also found in the pasture type. Two patches of skunk currants, containing a total of 2,300 feet of live stem and well screened by spruce and hardwood, were situated near the northwestern portion of the merchantable stand. No wild ribes were located in this stand, but small scattered bushes occurred nearby in the pasture and on rock outcrops in the adjoining mixed growth of conifers and hardwoods. The wild ribes were eradicated in 1925. A patch of cultivated black currants, probably *R. nigrum*, was grown on an adjoining farm for several years in an open situation about 800 feet west of the area studied. This currant garden was started about 1909 by a former resident, with a dozen plants imported from a nearby State. The planting was increased by propagation from cuttings until it contained about 80 large bushes, which were destroyed in 1917 by the present tenant, because he was unable to find a suitable market for the fruit. No other cultivated ribes were found, or are known to have existed, within 1 mile of the area.

FIELD METHODS

The data on blister rust damage in the area studied were obtained separately for the two types, because of the difference in character of growth resulting from the difference in stocking. The pasture

⁶ Figures for "live stem" represent the total linear measure of stem wood in living canes, branches, and spurs of the plant.

type was divided into twenty-five 1-acre plots, whereas the merchantable stand was studied as a unit. A careful search was made for ribes, and the location, species, and live-stem length were recorded. Detailed information was secured on all white pines, except seedlings less than 4 years old and trees dead from causes other than blister rust, principally suppression and cattle injury. The data obtained for each white pine included location, height, diameter at breast height, age, dominance, and condition of health as influenced by the blister rust. The heights were determined by ocular estimates, checked occasionally by actual tape measurements. Tree diameters were obtained with calipers, and tree ages by counting the yearly growth nodes. In taking the data on dominance, the trees were arbitrarily classified as suppressed or not suppressed, the latter classification including the three normal classes, namely, intermediate, codominant, and dominant. This grouping was chosen in order that trees of little or no value to the stand and those still important might be considered separately. The condition of health as influenced by blister rust was ascertained by climbing the trees and carefully examining them. Each tree was recorded as healthy or diseased. In the case of infected trees it was noted whether or not the disease had reached the trunk.

As long as the pine bark adjoining the canker or area directly affected by the disease remains alive, the blister-rust fungus continues to grow. Thus, cankers on branches frequently continue to extend until the trunk has been reached. The bark invaded by the disease eventually dies as the result of this parasitism. Soon after the canker has encircled the trunk, the diseased bark dies, girdling the tree and causing the death of that portion of the crown above the girdle. The fungus continues its downward growth until it reaches and girdles that section of the trunk below which there is no vigorous supporting foliage, thus killing the entire tree. In this study, white pines with infected trunks were further classified, on the basis of degree of decadence resulting from the disease, under three heads, namely, "top of crown alive", "top of crown dead", and "tree dead".

For the pasture type, the approximate age of the oldest infection was determined on each diseased pine, as there is no method of ascertaining exactly the year during which an infection originated. Since infection takes place through the needles (1), it is necessary to determine, from the number of annual nodes, the years during which the diseased portion of the tree bore needles. In this study the year of the infection was assumed to be the same as the year during which the infected internode was formed and first bore needles. Thus, the canker ages recorded are the maximum ages. In northern white pine the needles are normally retained during the second and sometimes the third year; therefore the actual age of an infection may be a year or two less than the recorded maximum.

DEVELOPMENT OF PINE INFECTION ON THE AREA STUDIED

The oldest cankers found on the area resulted from infections on internodes formed in 1908. These infections, however, may have occurred in 1909 or 1910 on needles originating⁷ in 1908. As several of the diseased trees in the pasture type were dead, the age of

⁷ RICHARDS, J. L. SUSCEPTIBILITY OF DIFFERENT AGED PINE NEEDLES TO BLISTER RUST AND RELATION BETWEEN THE NUMBER OF INFECTIONS ON PINES AND THE PERSISTENCE OF THEIR NEEDLES. U.S. Dept. Agr., Bur. Plant Indus. Blister Rust News 11: 241-247. [Mimeographed.]

the cankers that killed them could not be determined. Therefore, it is possible that the infections on 1908 internodes were not the earliest on the area. However, the disease was undoubtedly established there sometime between 1901, when it was first introduced into northern Vermont, and 1910. If the cankers on 1908 internodes were the first infection, the cultivated black currants planted about 1909 may have been responsible for establishing the blister rust in this locality (3).

Of the 2,226 pines on the entire area, 864 (39 percent) were found to be infected with blister rust (table 1). This represents conditions existing on the area when the studies were made, but undoubtedly many small pines had already been killed by the disease and had disappeared. This destruction of young trees is one of the most serious aspects of the damage caused by blister rust, and one of the most difficult to evaluate.

TABLE 1 —Relation of infection of white pines to length of period of exposure and the average height of the trees during this period

[Data are as of 1924 for the pasture type and 1925 for the merchantable stand]

Pines originating	Average height of pines ^a	Minimum years of exposure to possible infection from		Pines		
		Cultivated black currants	Wild ribes ^b	Total	Infected	
	Inches	Number	Number	Number	Number	Percent
Before 1910	—	7	13+	1,210	748	62
1910-12	30-71	5-7	11-13	93	31	33
1913-15	14-40	2-4	8-10	312	53	17
1916-18	7-55	0-1	5-7	545	32	6
1919-21	2-65	0	2-4	66	0	0
Total	—	—	—	2,226	864	39

^a Average heights of trees during period of exposure to infection from wild ribes were computed from tables by Spring (11)

^b While the actual period of exposure to infection from wild ribes probably extended from 1910 to 1924, inclusive, the years 1923 and 1924 are not considered in these data, because infections occurring in these years were not sufficiently developed to be found in 1924 when the pasture type was studied

The percentage of pines infected becomes greater with the increase in tree heights and in the number of years of exposure to infection (table 1). The quantity of pine foliage exposed to infection is an important factor influencing the amount of infection. On the basis of foliage volume alone, a large tree has a much greater chance of becoming infected than a small one. Height of tree is an imperfect index of the foliage volume, because among young trees the amount of foliage increases at a much greater rate than the height. However, since no published data are available on the rate of increase of foliage volume with age, the average heights are used in this case to indicate the trend of foliage volume. The fact that no infection was found on the 66 trees originating after 1918 may be the result of their small size or of the removal of the cultivated black currants in 1917.

There were three possible ribes sources for the infection on the area studied: The cultivated black currants that were grown for 7 years northwest of the area; the skunk currant patches located in

1924 just outside the northwest corner of the merchantable type; and the individual bushes, largely wedge-leaf gooseberries, found scattered through the pasture type, likewise in 1924. The ribes record is incomplete, as no definite information is available on the extent or distribution of the population of the two wild species during the infection period under consideration, namely, 1910 to 1922,

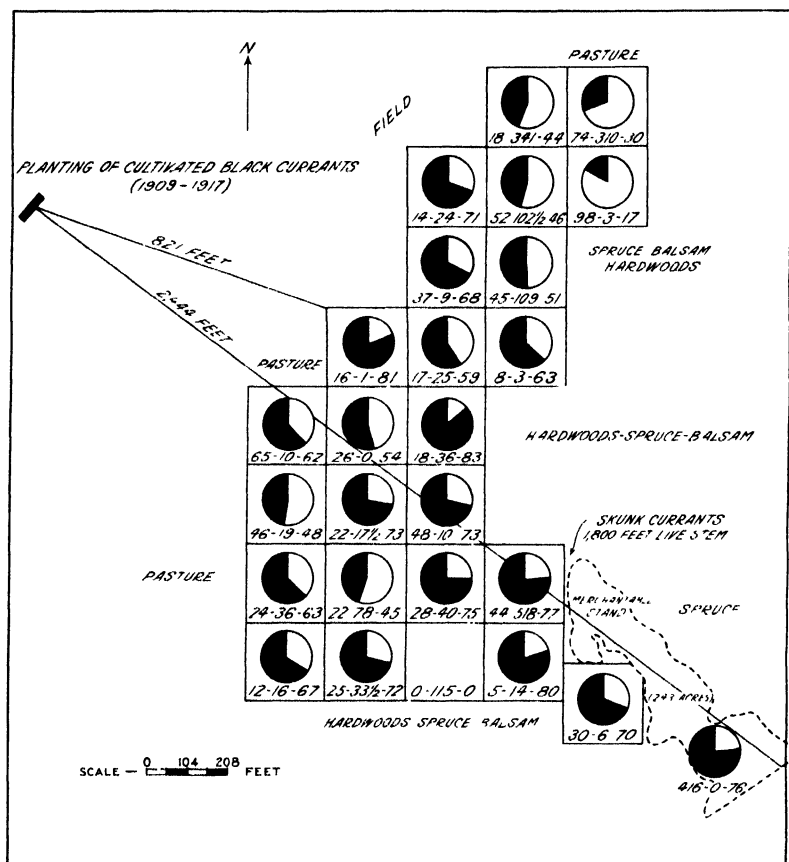


FIGURE 2 Amount and distribution of pines, ribes, and pine infections on the study area at Waterford, Vt. Only the 1,210 pines are included which originated before 1910 and were therefore exposed to the maximum period of infection. Each square represents a 1-acre plot in the pasture pine type. Broken line indicates boundary of merchantable pine stand. Reading from left to right, numbers separated by dashes in squares indicate, respectively: (1) Number of pines, (2) number of feet of ribes live stem, (3) percentage of pines infected. Shaded portion of each circle illustrates percentage of pines infected in respective plot.

inclusive. Several other factors, aside from the quantity and location of ribes, have undoubtedly influenced the distribution of pine infection among the individual units of the area shown in figure 2, especially the variation in foliage volume and screening of the pines and in topography. Independent of all other factors, in the ten 1-acre plots comprising the four northernmost tiers of those studied, the percentages of pines infected decrease as the distances from the cultivated black currant planting increase. This condition strongly indicates the importance of the ribes (probably cultivated black

currants) northwest of the area as the principal source of the disease in the northern portion. The inverse relationship of the amount of infection and the distance from ribes has been noted on other tracts (7). In the remainder of the Waterford area the influence of the cultivated currants is not clearly indicated by the variation of intensity of infection with distance. It seems probable that the wild ribes contributed materially to the infection found in the southern portion, especially in the vicinity of the skunk currant patches.

BLISTER RUST DAMAGE IN THE MERCHANTABLE STAND

The size of the white pines in the merchantable stand (fig. 3) prevented rapid disintegration of the trees killed by the blister rust and afforded an opportunity to obtain data on the progress of the disease. Moreover, there has been no individual variation in the period of exposure to infection among the trees in this stand. Of course there have been unmeasured changes in the heights, diameters, and crowns of the pines during this period. These variations are necessarily disregarded in the discussion.

The merchantable stand contained 416 white pines, of which 364 (88 percent) were classed as not suppressed (table 2). In 1925, 76 percent of the total pines were infected, 78 percent of the unsuppressed trees and 65 percent of the suppressed trees being diseased. The 1930 examination showed no change in these percentages, since no trees were found to have been initially infected during the period from 1925 to 1930. Especially noteworthy is the higher infection ratio among the pines not suppressed, the best trees in the stand. Undoubtedly, the smaller crowns of the suppressed pines reduced their chances of becoming diseased.

TABLE 2—*Dominance and blister rust infection of white pines in merchantable stand in 1925 and 1930*

Condition of health as affected by blister rust	Not suppressed				Suppressed				Total			
	Number		Percent		Number		Percent		Number		Percent	
	1925	1930	1925	1930	1925	1930	1925	1930	1925	1930	1925	1930
	1925	1930	1925	1930	1925	1930	1925	1930	1925	1930	1925	1930
Trees not diseased	80	80	22	22	18	18	35	35	98	98	24	24
Trees with branch cankers only	76	45	21	12	2	2	4	4	78	47	19	11
Trees with stem cankers												
Top of crown alive	152	123	42	34	10	3	19	6	162	126	39	30
Top of crown dead	32	43	9	12	10	4	19	7	42	47	10	11
Trees dead	24	73	6	20	12	25	23	48	36	98	8	24
Total	364	364	100	100	52	52	100	100	416	416	100	100

In 1930 only branch cankers were found on 47 white pines, or 11 percent of the total number, regardless of dominance. From the viewpoint of potential damage, the trees in this group may be regarded as ill, with a chance to recover. Pines are sometimes killed by severe pruning caused by numerous branch infections. This type of damage is, however, relatively infrequent in *Pinus strobus*, and it does not occur in the merchantable stand at Waterford. In this area the death of the trees results from cankers that girdle the trunks.

Since the blister rust fungus is wholly parasitic, a canker originating on a branch can reach the trunk only as long as living bark intervenes. The death of the branch, either as a result of natural pruning or of

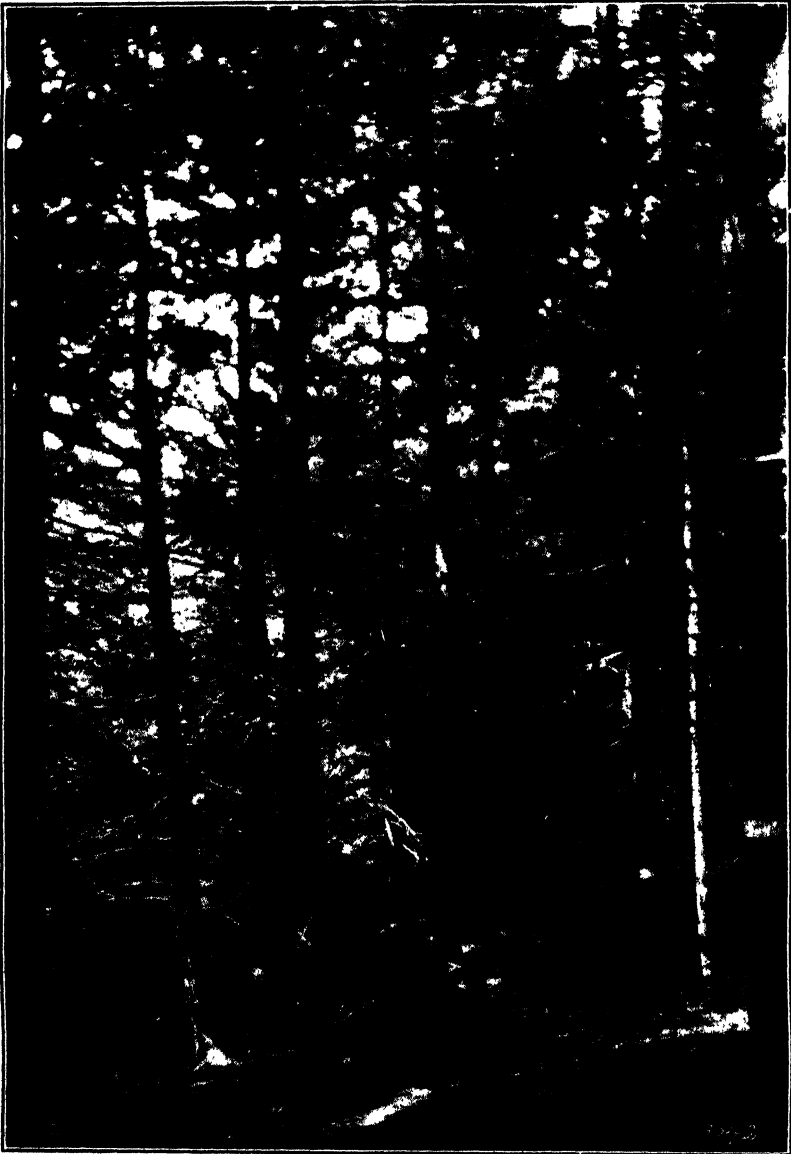


FIGURE 3.—Dominant, codominant, and intermediate white pines infected with blister rust in a merchantable stand at Waterford, Vt. Eleven pines, marked with white bands, have trunk cankers; two pines, marked with crosses (X), have only branch cankers. Photograph by E. C. Filler, 1926.

girdling by the canker itself, may cause the death of the bark between the canker and the trunk and thereby isolate the canker and kill it by starvation. However, a tree frequently has several branch cankers,

and usually one or more of them will reach the trunk. The progress of the disease in this respect is indicated by the fact that in 31 trees (40 percent of the 78 pines that had branch infections only in 1925) the cankers had reached the trunk by 1930 (table 2).

In 1930 trunk cankers were found on 271 white pines (65 percent of the total number). A trunk canker is almost invariably fatal to the tree, the only known exceptions occurring in the upper part of long terminal shoots that on rare occasions die before the fungus has had time to reach the node below. In the stand under discussion all the trunk cankers were located below the terminal shoot and may be considered fatal to the trees. Of the 271 trees with trunk cankers in 1930, 98, or 36 percent, had already been killed. It is only a question of time as to when the remaining 173 will die as a result of the disease. The rate of killing is indicated by the increase in the number of dead trees, from 36 in 1925 to 98 in 1930 (table 2).

The average annual progress of the disease in damaging this stand between 1925 and 1930 is shown in table 3 in percentages of the total number of infected trees. The rate at which these changes occur is by no means constant but increases or decreases, or even reverses its direction, with the increase in the time elapsing since the date of infection.

RELATION OF TREE SIZE TO AMOUNT OF INFECTION AND RATE OF KILLING

Infection may take place through needles borne on the trunks (9, p. 28) and is usually confined to that part of the terminal shoot bearing needles at the time of exposure. Occasionally, infection occurs through adventitious shoots on the trunk. However, trunk cankers usually result from the growth of the fungus into the trunk from points of infection on branches; this was the case in the merchantable stand at Waterford.

The time required by the fungus to reach the trunk through a branch is governed by its distance from the trunk and its rate of growth. Although pines with large crowns and many needles have a greater chance of becoming infected than do small ones, the average distance between the needles and the trunk is greater in large crowns than in small ones. Therefore, the fungus as a rule requires more time to reach the trunk in trees with the larger crowns. Rhoads (8) measured the development of cankers on trunks up to 4 inches in diameter and found that the rate of growth of the fungus increased with the diameter of the diseased part. Computations from Rhoads' tabulations show, nevertheless, that despite the increased rate the fungus required more than three times as long to completely girdle a stem in his largest-diameter class than in his smallest because of the disproportionate increase in circumference. This may partly account for the fact that 73 percent of the infected suppressed trees in the merchantable stand had died from blister rust by 1930, whereas only 26 percent of the diseased unsuppressed pines had been killed (table 3).

The status of the health of the pines in the merchantable stand in 1930, as influenced by the blister rust, is shown in figure 4, in which the data are arranged according to the height and diameter of the trees within each of the dominance groups. In considering this figure, it should be noted that the percentages of pines infected, represented by the solid-black column, are now fixed, since further infection of the

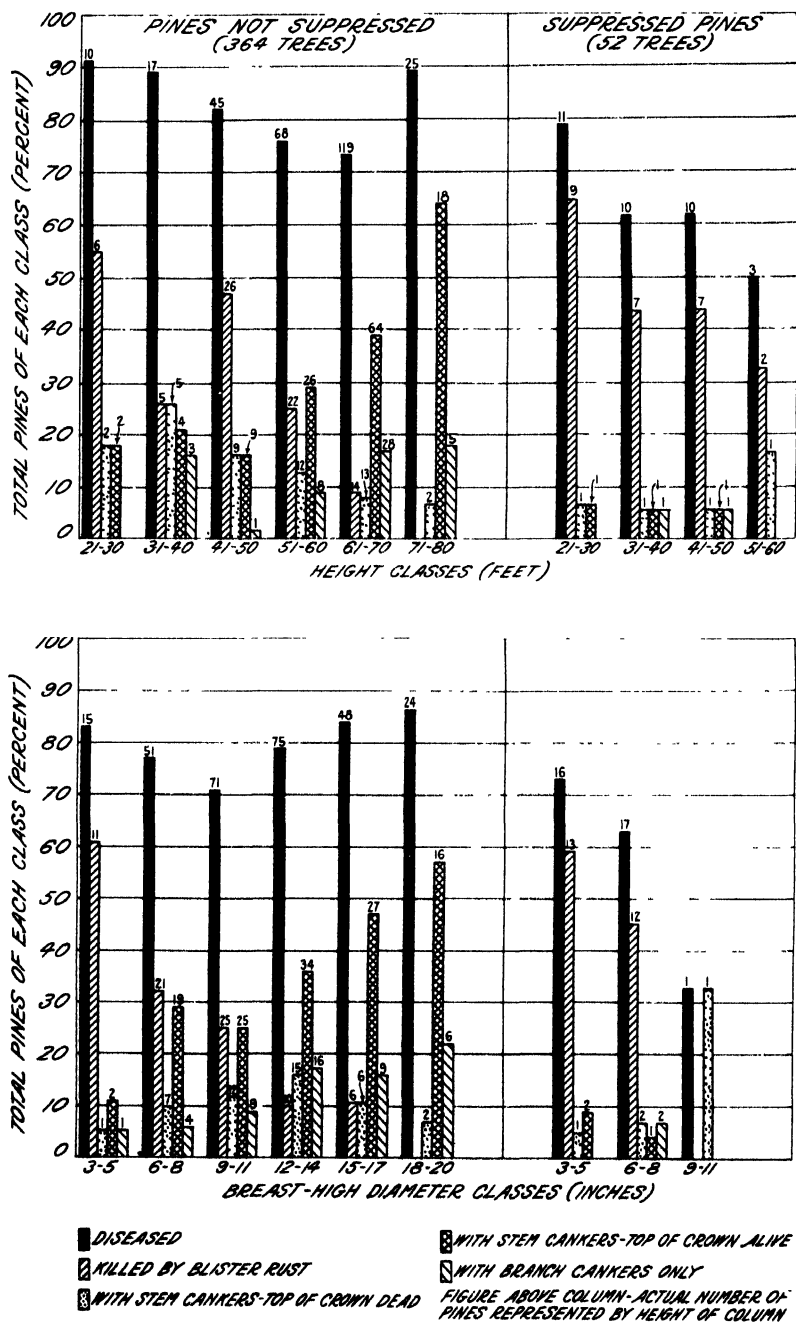


FIGURE 4—Status in 1980 of health of white pines in merchantable stand as influenced by blister rust. Data arranged according to height and diameter of trees within each dominance group

trees is being prevented by eradication of the ribes. All other columns, representing percentages of trees in the various damage classes as of 1930, are at temporary levels, which vary as the disease progresses. Unsuppressed pines of all sizes have been attacked by the rust (fig. 5), the number of trees infected ranging from 71 to 91 percent (fig. 4). Moreover, unsuppressed pines of all sizes, except the largest in each class, have been killed. (Forty-nine percent of the 73 dead

TABLE 3.—Changes in percentages of infected white pines in merchantable stand between 1925 and 1930

Condition of health as affected by blister rust	Not suppressed			Suppressed			Total		
	1925	1930	Average annual change	1925	1930	Average annual change	1925	1930	Average annual change
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Trees with branch cankers only	27	16	- 2 2	6	6	0	25	15	- 2 0
Trees with stem cankers									
Top of crown alive	54	43	- 2 2	29	9	- 4 0	51	39	- 2 4
Top of crown dead	11	15	+ 8	29	12	- 3 4	13	15	+ 4
Trees dead	8	26	+ 3 6	36	73	+ 7 4	11	31	+ 4 0

pines in the unsuppressed group were in height classes of 51 to 80 feet, and 56 percent were in diameter classes of 9 to 17 inches (fig. 4).

Since the time required for cankers to reach the trunk and kill the tree is influenced both directly and indirectly by the size of the crown

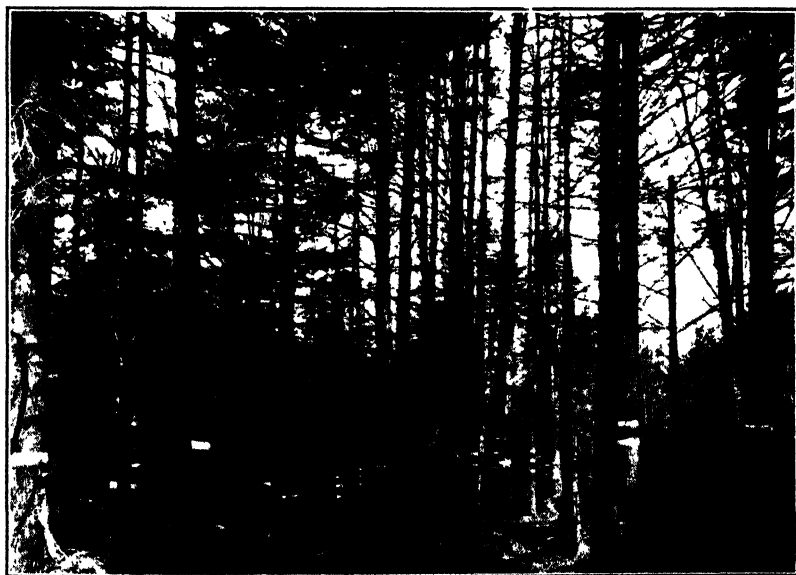


FIGURE 5.—White pines of all sizes infected and killed by blister rust in northwestern section of merchantable stand at Waterford, Vt. Degree of infection indicated by white markings as follows: 1 band, trunk canker (tree alive in 1930); 2 bands, killed by blister rust (tree alive in 1925, dead in 1930); 3 bands, killed by blister rust (tree dead in 1925); cross (X), branch cankers only, 1925 and 1930; cross and band, branch cankers only in 1925, trunk canker in 1930. Photograph by E. C. Filler, 1930.

and the upper portion of the trunk, both of which are closely related to the diameter of the tree at breast height, some correlation may be expected between diameter and the time required by the disease to kill the tree. When the effect of the disease is measured before the damage is completed, this correlation will be manifested by a downward trend in the proportion of trees killed as the diameter increases. This is the case in the merchantable stand at Waterford as of 1930, the proportion of trees killed by the disease decreasing as the diameter increases. It is evident that when the end of the damage has been reached, blister rust will have killed a majority of the pines in each of the diameter classes.

EFFECT OF BLISTER RUST ON BASAL AREA^a OF STAND

In 1930 the basal area of the white pines in the merchantable stand, including both the living trees and those killed by the disease, was 306 square feet (table 4), and of the hardwoods and spruce 236 square feet. The total basal area of 542 feet (223 feet per acre) is about normal for a fully stocked stand of this age and composition. Since the suppressed pines had a basal area of less than 10 square feet in 1930 (only 3 percent of the basal area of all the pines), they will not be considered separately in this connection.

TABLE 4.—*Basal area of white pines in the merchantable stand as of 1925 and 1930 as affected by the blister rust*

Condition of health as affected by blister rust	Basal area of white pines (square feet)		Percentage of basal area		Percentage of basal area of all species (1930) ^a
	1925	1930	1925	1930	
Trees not diseased	52	65	20	21	12
Trees with branch cankers only	54	45	21	15	8
Trees with stem cankers					
Top of crown alive	126	120	48	39	22
Top of crown dead	16	36	6	12	7
Trees dead	12	40	5	13	7
Total	260	306	100	100	56

^a No basal area or volume data were obtained in 1925 for species other than white pine

The proportion of the basal area of the white pines represented by trees beginning to die (top of crown dead) and those already dead as a result of the rust increased from 11 percent in 1925 to 25 percent in 1930, an average yearly increase of about 3 percent. The white pines in these two groups in 1930 comprised 14 percent of the total basal area of all species in the stand. Classifying as "doomed" all white pines with trunk cankers and as "may die" all white pines with branch cankers only makes it evident that the disease will eventually reduce the basal area of the white pines between 64 and 79 percent and the basal area of all species in the stand between 36 and 44 percent.

¹ The basal area of a tree is the area of a cross section of the stem at stump height inside the bark and is computed according to the formula: $b = \frac{\pi d^2}{4}$, in which b represents basal area and d the diameter of the cross section inside the bark. In this case, the diameter at breast height outside the bark was assumed to correspond with the diameter at stump height inside the bark.

EFFECT OF BLISTER RUST ON BOARD-FOOT VOLUME¹ OF STAND

In 1930 the volume of the white pines in the merchantable stand, including both the living trees and those killed by the disease, was 52,970 board feet, or 21,798 board feet per acre (table 5). The suppressed white pines contained but 1,036 board feet, or less than 2 percent of the total amount for this species. The volume of all other species was 21,644 board feet, or 8,907 board feet per acre, bringing the total contents of the entire stand up to 30,705 board feet per acre. This amount is below normal for a fully stocked mixed stand containing a large proportion of 60-year-old white pines. The difference may be partly due to the indeterminable loss in increment for that period during which each fatally diseased pine has suffered reduced or interrupted growth.

As indicated in table 5, the proportion of total pine volume represented by trees beginning to die (top of crown dead) and those already dead as a consequence of the disease increased from 8 percent in 1925 to 23 percent in 1930, an average increase of 3 percent per year. In 1930 the pines in these two classes contained 16 percent of the total board-foot contents of all species in the stand. The ultimate effect of blister rust will reduce the volume of the white pines between 64 and 80 percent, and the volume of all species in the stand between 46 and 57 percent.

TABLE 5. Board-foot volume of white pines in the merchantable stand as of 1925 and 1930, as affected by blister rust

Condition of health as affected by blister rust	Volume of white pines (board feet)		Percentage of volume		Percentage of volume of all species (1930)
	1925	1930	1925	1930	
Trees not diseased	8,453	10,693	19	20	14
Trees with branch cankers only	9,437	8,200	22	16	11
Trees with stem cankers					
Top of crown alive	22,160	21,904	51	41	30
Top of crown dead	2,280	6,132	5	12	8
Trees dead	1,518	6,032	3	11	8
Total	43,848	52,970	100	100	71

EFFECT OF BLISTER RUST ON STOCKING OF STAND

The merchantable stand, with 277 trees over 6 inches in diameter per acre, was about fully stocked before blister rust began to take its toll. Of these trees, 50 percent were unsuppressed white pines, 2 percent were suppressed white pines, and 48 percent were trees of other species, largely spruce. Between 239 and 284 of the 364 unsuppressed white pines on the 2.43 acres in the stand will eventually be killed by blister rust (table 2). This will leave between 80 and 125 unsuppressed white pines, or from 33 to 51 per acre. This great reduction in stocking is even more serious than these figures indicate, because of the irregularity of the original stocking.

The two $\frac{1}{4}$ -acre plots (fig. 6, A₁, B₁) are samples of the best pine stocking in the merchantable stand. On this half acre, there were 101 white pines and 38 spruce or hardwoods with breast-high diam-

¹ Volumes for pine, spruce, and hardwoods are based on tables prepared by Lyford and Margolin (4), Woolsey (5, p. 126), and Dana (2), respectively.

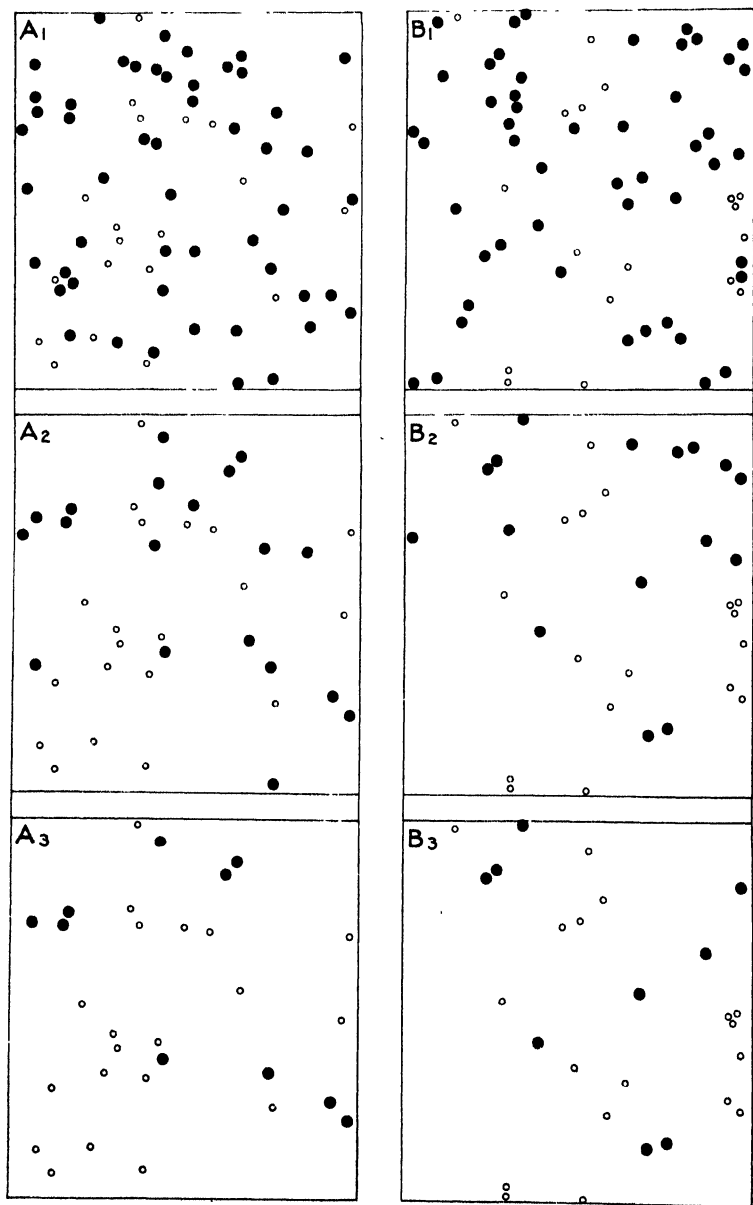


FIGURE 6—Effect of blister rust in reducing stocking on two 1/4-acre plots in merchantable stand. A₁ and B₁, two 1/4-acre plots, showing location of trees of all species whose breast-high diameters exceed 6 inches; A₂ and B₂, same as A₁ and B₁, omitting pines sure to die from blister rust; A₃ and B₃, same as A₂ and B₂, omitting also those pines which may die from blister rust. White pines whose breast-high diameter exceeds 6 inches are indicated by solid dots (●); spruce or hardwood whose breast-high diameters exceed 6 inches are indicated by smaller open circle (○).

eters greater than 6 inches. Blister rust will eventually reduce this stocking below that shown in A_2 and B_2 and will produce conditions approaching those shown in A_3 and B_3 . In addition to the trees shown in figure 6, the two $\frac{1}{4}$ -acre plots contained many small spruce and 12 suppressed pines with breast-high diameters under 6 inches. Seven of these pines are doomed as a result of trunk cankers, and one other has a branch infection that may eventually cause its death.

EVALUATION OF BLISTER RUST DAMAGE

Up to 1930 the economic loss caused by blister rust in the merchantable stand was relatively small. The pines killed by the disease on the 2.43 acres had a volume of 6,032 board feet (table 5). Blister rust does not injure the wood but may cause a constriction of the trunk in the portion covered by the canker. In the merchantable stand most of the cankers were above the commercial parts of the trees. However, it is doubtful if any of these dead pines could now be salvaged. Such trees deteriorate rapidly as a result of boring insects and wood-rotting fungi. The wood of these dead pines may, therefore, be considered a definite loss, amounting, at a normal stumpage value of \$8 per thousand, to \$48.26, or \$19.86 per acre.

The potential economic loss to this stand by blister rust includes all trees with trunk cankers and those with branch cankers that may die from the disease. The potential loss is, therefore, at least between 34,068 and 42,277 board feet on the 2.43 acres. It may even exceed this maximum figure by the amount of increment made by the diseased trees between 1930 and the time of their death. Moreover, these estimates of potential loss do not include the additional increment that would normally have occurred had the trees remained free of blister rust. Computed at a normal stumpage value of \$8 per thousand, the indicated potential loss amounts at least to between \$112.16 and \$139.18 per acre. This potential loss is the result of infections from ribs that were eradicated 5 to 13 years before the 1930 examination. Had these ribs been allowed to remain, potential loss would in all probability have approached 100 percent for the white pines in the stand.

The economic loss in this stand as a result of the disease may be kept at the present figure by immediately logging the entire stand. This may not, however, be practical, because of the small area and the condition of the lumber industry. Consequently, the loss continues to mount, approaching the highest potential-damage figure.

The economic loss from blister rust on this merchantable stand is far above the average for tracts of white pine of this size and age within the range of this species in the East. However, merchantable trees fatally attacked by the disease can be found in considerable numbers as scattered individuals or small groups throughout the white pine regions of New England and New York. This particular area illustrates the potential destructiveness of the disease to unprotected merchantable stands. Frequently, the damage is not recognized by pine owners until it is too late to utilize the diseased trees. Even in cases where the owners are aware of the increasing damage, the difficulty of detecting infected trees, particularly those with only branch cankers, and the additional cost of making selective cuttings may prohibit the salvage of white pines attacked but not yet killed by blister rust.

SUMMARY

This paper presents data on the occurrence and effect of blister rust on white pine in an area at Waterford, Vt., especially in a stand of merchantable timber.

A patch of cultivated black currants existed for at least 7 years, beginning about 1909, within a few hundred feet of the area. These cultivated bushes appear to have been an important source of infection. Wild ribes also participated in the spread of disease on the area. The cultivated black currants were destroyed in 1917 and the wild ribes in 1925. The earliest infections occurred between 1908 and 1910.

Data on the 2,226 pines on the entire area, grouped according to tree height and number of years of exposure to infection, show that the percentage of pines infected ranged from 0 to 62 percent, the proportion becoming greater with the increase in height and in number of years of exposure to the infection.

The merchantable stand was composed of a mixture of spruce, white pine, and hardwoods, about 60 years old. In 1930, 76 percent of the 416 white pines in this stand were infected, and 65 percent were doomed as a result of trunk cankers. Twenty-four percent of the total pines had already been killed. The percentage of diseased pines killed increased from 11 percent in 1925 to 31 percent in 1930, an average yearly increase of 4 percent.

The proportion of infected trees was greater in the unsuppressed group than in the suppressed group, probably because of the larger individual crowns in the former class. However, partly, at least, because of their smaller size, the suppressed pines were being killed more quickly than those not suppressed.

Pines of all height and diameter classes represented had been attacked by the rust, and in each class, except the largest-size group, some of the trees had already been killed.

The proportion of the basal area of the white pines represented by trees beginning to die or already killed by the rust increased from 11 percent in 1925 to 25 percent in 1930, an average yearly increase of about 3 percent. The killing of trees by the disease will eventually reduce the basal area of the white pines between 64 and 79 percent and the basal area of all species between 36 and 44 percent.

The proportion of the total board-foot volume of the white pines represented by trees beginning to die or already dead from the disease increased from 8 percent in 1925 to 23 percent in 1930, an average yearly increase of 3 percent. Ultimately, the volume of the white pines will be reduced between 64 and 80 percent and the volume of all species between 46 and 57 percent as a result of the disease. Had not the further spread of infection been stopped by the removal of the ribes 5 to 13 years before the 1930 examination, the ultimate loss of white pines would have approached 100 percent.

When damage from the disease has reached its maximum an average of between 33 and 51 unsuppressed white pines per acre will remain. This pronounced reduction in stocking is rendered still more serious by the unevenness of the original stocking.

The actual economic loss in the merchantable stand from blister rust, computed at a normal stumpage value of \$8 per thousand, amounted to \$19.86 per acre in 1930. As additional diseased white

pinus die or deteriorate, the loss will increase toward a maximum of at least between \$112.16 and \$139.18 per acre, depending on the period of delay in logging the stand.

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CYTOLOGY AND BREEDING OF *RUBUS MACROPETALUS*, THE LOGAN, AND RELATED BLACKBERRIES¹

By GEORGE M. DARROW, *senior pomologist, Division of Fruit and Vegetable Crops and Diseases*, and A. E. LONGLEY, *associate botanist, Division of Genetics and Biophysics, Bureau of Plant Industry, United States Department of Agriculture*²

INTRODUCTION

The highest dessert quality among dewberry and blackberry varieties appears to be found in a group derived wholly or in part from *Rubus macropetalus* Dougl. and related species of wild trailing blackberries that occur in the western parts of California, Oregon, Washington, and British Columbia. Among the high-quality varieties in this group are the Logan, Young, and Ideal Wild or Santiam. All are characterized by excellent flavor and a relative absence of seediness as compared with other varieties of blackberry. The flavor is strongest in the Logan and mildest in the Young, and suggests the flavor of the red raspberry more than that of the eastern American or European forms of blackberries that have been studied. Because of the great value of this group in breeding for high-quality varieties, this study of their cytology and relationship was begun.

MATERIALS

This investigation was carried on chiefly at Corvallis, Oreg. There were available for study several hundred seedlings of the Logan; also many Logan $\times$ Lucretia, Logan $\times$ Young, and Logan $\times$ Mammoth crosses made by C. E. Schuster, formerly of the Oregon Agricultural Experiment Station. There were also at the Oregon station the Ideal Wild or Santiam, Mammoth, Cory, Young, and Lucretia varieties, all of which, except the Lucretia, are derived wholly or in part from these western species.

Material of the wild *Rubus macropetalus* was obtained from fence rows near the station and from other places. Material of a cross of *R. macropetalus* $\times$ Logan, made by J. W. Ware, was obtained from western Washington. Other reported crosses of *R. macropetalus* $\times$ Logan were examined at Ware's ranch in western Washington and near Monroe and Florence, Oreg. Still other crosses of the wild *R. macropetalus* with various bramble varieties have been observed from time to time. The wild forms were studied at many points in

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² The writers express their appreciation to the workers at the Oregon State Agricultural Experiment Station, Corvallis, Oreg., for many courtesies and assistance in these investigations, also to S. F. Blake, Division of Botany, Bureau of Plant Industry, L. H. Bailey, Ithaca, N. Y.; and W. L. Jepson University of California, for the reading and criticism of the manuscript.

western Washington, Oregon, and California, from sea level to altitudes of about 5,000 feet in the Cascades, and to some extent in British Columbia.

The material for cytological preparations was collected at the experimental plots of the Oregon State College farm and from the wild in several nearby localities and at various points along the coast of California. Buds that had apparently developed to the stage when pollen mother cells were in the meiotic phases were picked and put at once into Carnoy's killing fluid. After a half hour the fluid was poured off and the buds were covered with absolute alcohol. Acetocarmine smear preparations of the pollen mother cells were then made.

PACIFIC COAST SPECIES OF RUBUS

CHARACTERIZATION

The western trailing blackberries differ from eastern species of dewberries and blackberries in being chiefly dioecious, with the staminate flowers usually much larger than the pistillate ones; in having leaves with leaflets—usually 3, often 5 in vigorous plants, but rarely 1—generally pinnate; and in having shoots and canes more or less glaucous. Western forms have 42, 35, 28, and 21 chromosomes, whereas most eastern trailing forms have 21. The western forms have much smaller drupelets and seeds than the eastern trailing species.

The western trailing blackberries are here considered as consisting of 3 species, although 5 or more species have been recognized by systematists. The following have been referred by Rydberg (10)³ to the section *Ursini* of *Rubus*:

Rubus ursinus Cham. and Schlecht., in *Linnaea* 2: 11. 1827.

R. vitifolius Cham. and Schlecht., in *Linnaea* 2: 10. 1827.

R. helleri Rydb., ex Brit. and Brown, North Amer. Flora 22: 460. 1913.

R. eastwoodianus Rydb., ex Brit. and Brown, North Amer. Flora 22: 460. 1913.

R. macropetalus Dougl., ex Hook., Fl. Bor. Amer. 1: 178. 1832.

Rydberg separates *Rubus eastwoodianus* and *R. macropetalus* from *R. ursinus* largely because of the glabrous fruit and sparingly pubescent leaves of the first two; and *R. vitifolius* and *R. helleri* from *R. ursinus* because of the sparingly pubescent leaves and stems of *R. vitifolius* and *R. helleri*, although all three species have pubescent fruit.

Bailey (4) in his latest monograph describes three species of the wild trailing blackberries of the Pacific region, namely, *Rubus ursinus*, *R. macropetalus*, and *R. eastwoodianus*. *R. ursinus* is described as a nonglandular tomentose or hairy species and *R. macropetalus* and *R. eastwoodianus* as glabrous or glabrescent, *R. macropetalus* being glandular and *R. eastwoodianus* nonglandular. Bailey does not recognize *R. vitifolius*, because it is represented by a description only, with no type specimen, and because he has found no *Rubus* which would answer the original description. Rydberg's description of *R. eastwoodianus* answers the original description of *R. vitifolius* closely, and according to Bailey (4, p. 305), it was established on two specimens collected in the region from which *R. vitifolius* was collected and described.

The writers' collections and studies appear to justify the establishment of three species, which include the important forms on the

³ Reference is made by number (italic) to Literature Cited, p. 330.

Pacific coast. Rare forms may still be found which will necessitate the establishment of additional species, but those examined seemed to represent crosses between overlapping species.

The three Pacific coast species are *Rubus macropetalus* Dougl., *R. ursinus* Cham. and Schlecht., and *R. loganobaccus* Bailey (2, 3). *R. macropetalus* is by far the most abundant, being the only species found throughout western British Columbia, Washington, and Oregon. Its southern range extends into the highlands of California. *R. ursinus* and *R. loganobaccus* are confined chiefly to the lowlands of a narrow belt of the coast of northern and central California.

Rubus macropetalus in Oregon and Washington is distinct from the two California species, having in general thinner, relatively longer, and more deeply serrate leaves. It is less hairy and more glandular than *R. ursinus* and has much slenderer and more trailing canes than *R. loganobaccus*. *R. loganobaccus* is separated from *R. ursinus* and from *R. macropetalus* by its much greater stature and by the larger size of most of its parts.

Rubus loganobaccus was described by Bailey as a species on the basis of the horticultural variety Logan, of which no wild form was known. In the writers' survey, however, closely related forms were found over a north and south range of some 400 miles. They were especially abundant in the region near Eureka, Calif., constituting the only wild forms in some large areas there. Because they are such giant forms as compared with *R. macropetalus* and *R. ursinus*, they clearly represent another species. Where this species comes in contact with *R. macropetalus* north of Eureka, intermediate forms were observed; likewise, forms intermediate between *R. loganobaccus* and *R. ursinus* were collected south of San Francisco, especially near Watsonville. That the wild *R. loganobaccus* originated from natural crosses of the Logan with *R. ursinus* is untenable, because (1) the observed crosses of the Logan with *R. macropetalus* (similar in size to *R. ursinus*) are not giant in stature but are intermediate, (2) the wild forms cover too great an area, and (3) in the herbarium of the University of California is a collection of the giant form (no. 372722 from Sonoma County), which was made in 1881, the year when the seed from which the Logan originated was said to have been planted.

The following description of *Rubus loganobaccus*, based on wild plants, includes its essential specific characteristics.

Rubus loganobaccus Bailey.—Primocanes⁴ erect to 2.5 m, then arching and trailing, stout (0.7 to 1.0 cm or more in diameter), usually glaucous, pubescent; prickles many, long, short, or both, straight or curved, rather weak, with enlarged base; leaves evergreen, glabrate to gray tomentose below, unequally serrate, those of primocanes 3- to 5-pinnately foliate on prickly tomentose petioles, leaflets ovate to triangular-ovate, terminal one on a petiolule 2 to 3.5 cm long, sometimes obscurely to prominently lobed, lateral leaflets with very short petiolules (½ cm). Floricanes⁵ with 6 to 15 or more flowers on leafy shoots, pedicels sometimes glandless, usually with some glandular hairs, sometimes with many glandular hairs, prickly, tomentose; flowers usually staminate or pistillate; petals of pistillate flowers small to about 1 cm long, those of staminate flowers much longer to about 2 cm long; calyx tomentose, bristly, sometimes prickly; fruit oblong or conical, sweet, usually 1 inch or less long, blackish, glabrous or pilose.

Habitat.—Fields, valleys, and hills of the coast of northern and central California, from about 40 miles north of Eureka south to Carmel, approximately within the redwood area.

⁴ First-year shoots.

⁵ Second-year bearing canes.

The Logan differs from the wild *Rubus loganobaccus* in its hermaphrodite flowers and red fruit but is not more vigorous.⁶ The Logan may be considered a glandless form with leaves gray tomentose below.

Rubus ursinus is here considered the coastal representative of *R. macropetalus* in California, differing from the latter in having the leaves usually thicker, more pubescent, and less sharply and deeply serrate; canes and inflorescence pubescent; few or no glandular hairs on the inflorescence; and fruit usually pilose. To the north and in the mountains *R. ursinus* varies toward *R. macropetalus*; in Oregon and Washington, forms of *R. macropetalus* vary toward *R. ursinus*. Typically, however, the two species are quite distinct. At Carmel a form was collected that probably should be considered a variety (*monophyllus*)⁷ of this species, having most of the leaves entirely or slightly lobed. Throughout the coast region north and south of San Francisco may be found forms that differ from the type in one or more characteristics. Some are glabrate, others glandular, and others slightly to deeply and unequally serrate.

Rubus macropetalus is relatively distinct except as it varies toward *R. ursinus* and *R. loganobaccus* in western California. It is essentially a glabrous, deeply serrate thin-leaved form having slender glaucous canes with glandular hairy inflorescences. Eastward in Idaho and southward in central and southern California it varies considerably in leaf characters from this type. In any one region, moreover, it remains a variable form.

In western Oregon and Washington, and in California in the same locality, may be found forms of *Rubus macropetalus* ranging from pubescent to nearly glabrous and having canes ranging from stout to slender, prickles ranging from few to many, and leaves ranging from pubescent to nearly glabrous and from pointed to obtuse. The inflorescence ranges in hairiness from slightly glandular to quite glandular, occasionally very glandular, and in size of clusters from large to small. The berries range from long to short, from rather firm to soft, and are usually purplish black but occasionally red or white.

On Bainbridge Island, Wash., a nearly thornless plant was seen, and at Puyallup, Wash., a completely thornless one. Shade forms have fewer thorns than those growing in open fields. The shape of leaves on the same plant may vary greatly according to whether the cane grew in early or in late summer. The early summer forms have much longer, more pointed leaves; the later canes, less serrate, shorter leaves. The leaves persist until midwinter and often throughout the winter, especially in protected places.

Near the summit of the Coast Range near Triangle Lake, Oreg., several hermaphrodite plants were found. One plant there produces male flowers in the spring but is hermaphrodite when flowering in the fall. Though normally having dark wine-purple fruit, forms with large red fruit have been selected for cultivation. Forms are reported that produce berries as large as the Logan.

This species is found chiefly in open woods, fields, and along roadsides. The finest berries are produced by bushes growing in moist,

⁶ George F. Waldo in a letter dated July 6, 1933, states that he found wild hermaphrodite plants of *Rubus loganobaccus* near Eureka, Calif.

⁷ *Rubus ursinus monophyllus*, var. nov. Folia maxima ex parti integra vel leviter trilobata interdum trifoliolata. Carmel, Calif., May 8, 1932, G. M. Darrow. Type in U.S. National Herbarium, no. 1586198.

partially shaded locations. Where forest fires are not too severe, the tangled cane growth often comprises the principal part of the vegetation of cut-over land for a few years, until the undergrowth and trees become large enough to kill it with excessive shade. In favorable seasons it bears fruit of fair size in great abundance. East of the Cascade Mountains and south in California it is found chiefly in the moist ravines. As it flowers very early in the spring, frosts and rains are considered to be responsible for the small size and the poor set of the fruit in some years. In an open field or by the roadside (fig. 1) the mature plants of this species are hardly 2 to 3 feet high, but where they climb over brush or stumps their canes may be many feet in height.

CHROMOSOME STUDIES

Chromosome material was secured from male plants of the dioecious form of *Rubus macropetalus*, common in the wild, and from the perfect-flowered cultivated form known as the Ideal Wild variety.

In material collected from four distinctly separate localities in Oregon, the haploid chromosome number of male plants of the



FIGURE 1 —Wild *Rubus macropetalus*, showing trailing habit.

dioecious form of *Rubus macropetalus*, common in the wild, was found to be 42. This number is the highest that has yet been reported for a *Rubus* species and it seems significant that this high number is associated with dioeciousness.

Figure 2, C, shows a heterotypic metaphase from material collected 10 miles from Corvallis, Oreg., on the Newport road. Figure 2, B, shows a homotypic metaphase from material collected on the summit of the highway 40 miles south of Roseburg, Oreg. The figures in this wild material were frequently very clear, and the regular behavior of the chromosomes made accurate counting possible in spite of the large number.

The haploid chromosome number of male plants of *Rubus loganobaccus* collected from the wild in two widely separated localities was 21. One of these localities, about 42 miles north of Eureka, Calif., was the northernmost point at which this species was found. The other locality, where two collections were made, was Aptos, Calif., about 350 miles southward. Plants collected from a third locality, Scotia, Calif., nearly 30 miles south of Eureka, had 35 chromosomes.

In a fourth collection, at Carmel, Calif., the most southern station for this species, the plants had 28 chromosomes. Material of a form closely resembling *R. macropetalus* and collected near the *R. loganobaccus* plants at the northernmost station, 42 miles north of Eureka, also had 28 chromosomes. It seems probable that the chromosome number of *R. loganobaccus* is 21.

The haploid chromosome number of the Ideal Wild variety is 28. Figure 2, A, shows a typical heterotypic metaphase of this form. The 28 chromosomes can be counted in the early meiotic phases, since univalent chromosomes were rarely seen. This variety originated

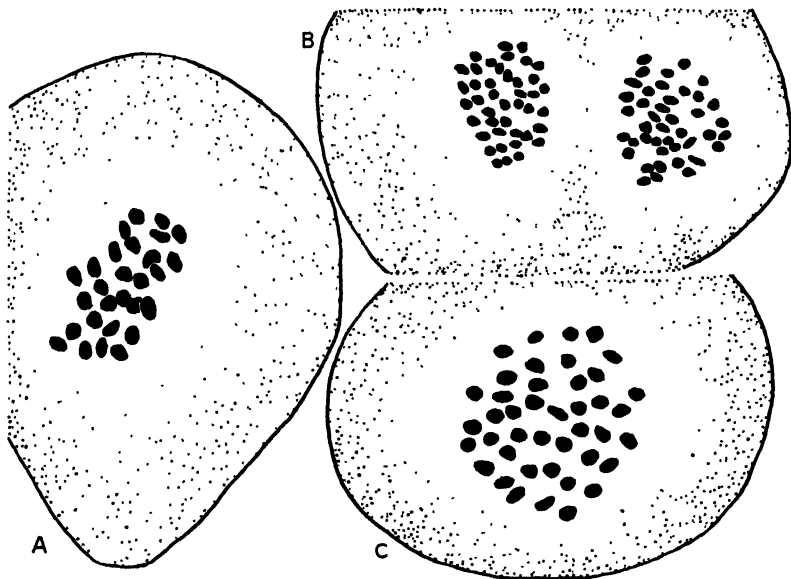


FIGURE 2—Chromosomes in *Rubus macropetalus* and related form. A, Heterotypic metaphase in the perfect-flowered form, Ideal Wild, B and C, homotypic and heterotypic metaphase, respectively, in *R. macropetalus* from the wild.

in a garden in Salem, Oreg., and although it resembles *Rubus macropetalus*, its hermaphrodite flowers and somewhat tomentose leaves suggest that it originated from a *R. macropetalus* × Logan hybrid.

THE LOGAN

HISTORY

Kinney (8), Darrow (7), Backhouse (1), and others have discussed the origin of the Logan. According to reports by the originator, J. H. Logan, of Santa Cruz, Calif., in 1881 he planted seed of the Aughinbaugh, an extremely vigorous pistillate selection of the wild *Rubus*, but having larger, darker leaves, and fruit similar to the Logan in shape. This plant grew in Logan's garden near the Texas Early blackberry and a red raspberry similar to the Red Antwerp. The resultant seedlings, with one exception, were said to be similar in appearance. The exceptional plant later was named the Loganberry. The majority of the seedlings were considered crosses of the Aughinbaugh and the Texas Early blackberries. The Mammoth blackberry

was selected either from this first lot or from later ones and was similar to the majority of the seedlings. Seedlings other than the Logan and the Mammoth were distributed but were said to be pistillate and were later discarded. The Logan was perfect-flowered and red-fruited and was considered a cross of the Aughinbaugh blackberry and the Red Antwerp raspberry. Logan stated that seedlings of the Logan which he later raised were similar to it, having red, mostly small fruit, and that some hundreds of crosses of the wild blackberry with the Logan, Mammoth, and Black Logan were perfect-flowered but sterile.

In 1916 Backhouse (1) questioned the hybrid origin of the Logan because it did not show segregation but resembled in breeding behavior a variety of a true species.

Crane and Darlington (5) studied the cytology and inheritance of the Logan. They found that it had 21 chromosomes. Selfed seedlings showed segregation of characters for glaucous and nonglaucous shoots in a 3 to 1 ratio. Other characters were not sufficiently distinct to determine ratios. These workers also studied the Laxton berry, a cross of the Superlative (a red raspberry) $\times$ Logan, and a sister plant. Each had 49/2 chromosomes and showed segregation of characters in the seedlings. They concluded that the Logan was of hybrid origin, that it may have originated from an octoploid (28) blackberry crossed with a tetraploid (14) raspberry, and that because of the greater proportion of blackberry chromosomes, the raspberry characters were largely lost. They further stated that such a form would fail to segregate the characters of the parents because the pairing of the chromosomes of the two parents would lead to irregularities in division and to the segregation of entirely new types whose vitality would usually be reduced. They stated that this actually occurs in the Logan, "such a large proportion of whose offspring can only be described as defective."

BREEDING AND CYTOLOGY

LOGAN SELFED

The several hundred selfed seedlings of the Logan which have fruited at the Oregon Agricultural Experiment Station showed the variations that might be expected among seedlings of a variety of a true species. Nearly all seemed about as vigorous as the Logan and many were much more vigorous. However, a large number of dwarfs were destroyed because they resembled plants affected with the "dwarf" virus disease. The various seedlings ripened from a week earlier to approximately 2 weeks later than the Logan; several bore larger fruit, but nearly all bore smaller fruit; some were better flavored than the Logan, but many were more acid; less than 1 percent bore fruit having the calyx attached when picked; none separated from the receptacle as in the raspberry; less than 1 percent bore glabrous fruit; none of the several hundred were entirely sterile, most being very productive. In cane, leaf, and fruit there was no suggestion of the raspberry. Of more than 400 seedlings, all or nearly all, were productive and were not defective as reported by Crane and Darlington (5).

Table 1 shows data on the fruit and seed of the Logan as compared with those of Logan seedlings and other varieties. The measurements were made on 10 berries of each variety. The seed of the Logan is shown to be much larger than that of the Cuthbert red raspberry or that of *Rubus macropetalus*.

TABLE 1.—Fruit and seed of the Logan as compared with those of Logan seedlings, four other blackberry varieties, and the Cuthbert red raspberry

[Data represent averages of measurements on 10 berries]

Variety	Weight of berry	Drupelets per berry	Seed weight
	Grams	Number	Milligrams
Logan	7.22	98.6	2.55
Logan seedlings	2.38-5.72	58.1-96.6	1.80-2.99
<i>Rubus macropetalus</i>		54.9	1.39
Ideal Wild	2.30	44.6	1.67
Young	8.34	73.8	3.82
Mammoth	5.88	96.0	2.32
Cuthbert red raspberry	2.80	85.2	1.43

It is worthy of note that several variations have been observed in fields of Logan blackberries in Oregon. In some cases the variant plants came into leaf earlier than the rest; in others, the canes were less glaucous. Their appearance suggested that they had originated

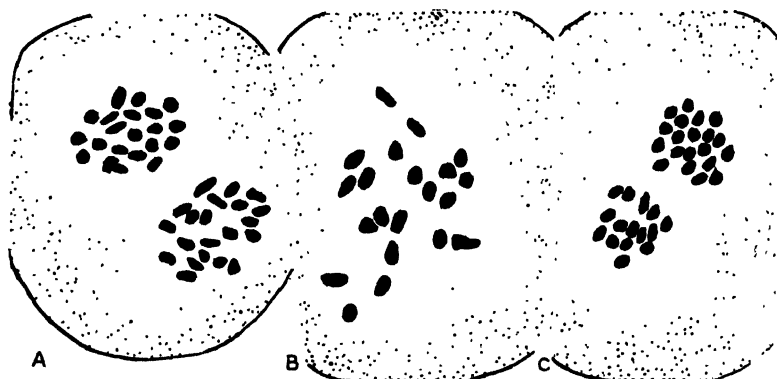


FIGURE 3.—Chromosomes in the Logan and in selfed Logan seedlings. A, Homotypic metaphase in the Logan; B, prophase in a hexaploid Logan seedling (no. 14); C, homotypic metaphase in a pentaploid Logan seedling (no. 3).

as seedlings of the Logan but bearing similar fruit. One Logan plant had produced some thornless (but fruitless) canes that were obviously bud sports.

The fruit clusters of the Logan were compared with those of its seedlings. Forty-eight clusters of the Logan had an average of 7.9 berries, whereas averages of 10 clusters each of 30 seedlings ranged from 3.7 to 10 and averaged 6 berries. Of 409 seedlings 269 had glaucous and 140 glabrous canes, approximating a 2 to 1 ratio. Crane and Darlington (5) listed 40 seedlings having waxy canes and 13 devoid of wax, which suggested a 3 to 1 ratio.

The haploid chromosome number of the Logan is 21. This number for a clonal offspring of the original plant agrees with that given by Crane and Darlington for seedling offspring. The chromosomes of the Logan were generally found to pair regularly, and the reduction phases show only occasional irregularities in chromosome distribution. Figure 3, A, is a typical homotypic metaphase in which 21 chromosomes are shown on each plate.

The chromosomes of 11 seedlings of the Logan, selected at random, were examined; 10 of these had the same chromosome number as that of the parent plant. Figure 3, B, shows a prophase stage from plant 14. At this phase the chromosomes frequently show variations in size and shape, but are small and do not lend themselves to a detailed morphological comparison. The chromosome behavior during meiosis of the pollen mother cells in the 10 hexaploid seedlings is generally as regular as that found in the mother plant. Occasionally preparations from a few of the plants showed unpaired chromosomes at diakinesis and random distribution of these univalents if they did not divide in the heterotypic division. If they divided, the halves went at random to the poles in the homotypic division.

The eleventh plant (no. 3 in table 2) of this small group of seedlings was found to be pentaploid. The chromosome behavior of the plant is distinctly more irregular than that found in any of the 10 sister plants. There were always some unpaired chromosomes in the prophase of the heterotypic division, and irregularities in chromosome distribution were prevalent in both divisions. Occasionally, chromosomes were extruded into the cytoplasm during the heterotypic division. Figure 3, C, shows a homotypic metaphase with 15 chromosomes on one plate and 20 on the other. Figures at this phase frequently show such unequal numbers; in some cells there are minor spindles around chromosomes previously extruded into the cytoplasm.

Table 2 shows the character of the pollen in the plants used in this study. The hexaploid Logan seedlings have a low percentage of sterile pollen. The pentaploid plant has approximately 50 percent of its pollen grains aborted.

TABLE 2.—Chromosome number and pollen counts in the Logan variety, Logan seedlings, hybrids, and related forms

Variety or species	Seedling no.	Number of chromosomes	Number of pollen grains in condition indicated	
			Good	Poor
Logan.....		21	133	5
	3	35/2	111	90
	4	21	480	180
	5	21	135	38
	7	21	104	11
	8	21	140	11
Logan seedling.....	10	21	152	21
	11	21	110	4
	12	21	105	10
	13	21	114	18
	14	21	133	15
	16	21	118	14
Mammoth.....		21	239	98
	4	35/2	0	100
	5	21	117	8
	6	21	44	100
Logan × Mammoth.....	7	35/2	(a)	(b)
	8	21	127	18
	10	21	132	12
	12	21	203	7
	13	21	56	314
Young.....		21		

^a Less than 10 percent.

^b More than 90 percent.

TABLE 2.—*Chromosome number and pollen counts in the Logan variety, Logan seedlings, hybrids, and related forms—Continued*

Variety or species	Seedling no.	Number of chromosomes	Number of pollen grains in condition indicated	
			Good	Poor
Logan × Youne	1	21	(a)	(b)
	2	21	128	21
	3	21	112	168
	4	21	122	38
	5	21	127	37
	6	21	146	77
	6A	21	122	72
	7	21/2	9	114
	8	21	130	12
Lucretia	9	21	151	25
		21		
	1	21	100	80
	2	21	108	78
Logan × Lucretia	3	21	104	31
	4	21	139	103
	5	21	100	106
	6	21	104	36
	8	21	124	16
	12	21	120	90
	13	21	108	213
Phenomenal Cory		21		
<i>Rubus macropetalus</i>		12	126	2
Ideal Wild		28	106	19

^a Less than 10 percent^b More than 90 percent.

Earlier studies of *Rubus* (9) showed that forms with an even multiple of the basic chromosome number 7 are much more fertile than those with an odd multiple. Crane and Lawrence (6) in their study of *Rubus* have confirmed this relationship between the chromosome number and the fertility of the pollen.

The results of the pollen study of the few representative Logan seedlings are in agreement with these earlier studies, the pentaploid being the only plant showing a high percentage of sterile pollen.

LOGAN × MAMMOTH HYBRIDS

The Mammoth originated about the same time as the Logan but was considered a hybrid between Aughinbaugh, a variety of *Rubus ursinus*, and Texas Early blackberry (close to *R. argutus* Link × *R. enslenii* Tratt.). It is an even more vigorous plant than the Logan. Its canes and thorns are much larger and suggest to some extent the eastern blackberry. Its pollen showed a relatively high percentage of sterile grains (29 percent as compared with 4 percent for the Logan and 2 percent for *R. macropetalus*). Of 164 crosses of Logan × Mammoth only 2 were at all productive and these did not set fully; the remainder were entirely or nearly sterile. There were no contrasting characters on which to base a study of inheritance.

The Mammoth, like the Logan, was found to have 21 as its haploid chromosome number. Figure 4, A, shows a homotypic anaphase of Mammoth, and figure 4, B, shows a homotypic metaphase from a sport of Mammoth known as the Cory. Both figures show clearly the hexaploid chromosome number.

Eight F₁ hybrids between Logan and Mammoth were studied. Six of these have 21 as their haploid chromosome number. The chromo-

somes in some of these hexaploid plants were frequently unpaired in the early meiotic phases, and irregularities in chromosome distribution of later phases indicated the presence of incompatible chromosome sets. Figure 4, C, is a heterotypic prophase from one of these plants showing 20 bivalent and 2 unpaired chromosomes. Figure 4, D, shows a homotypic anaphase from a sister plant. This phase was found to be the most satisfactory for obtaining accurate chromosome counts if the chromosomes of the four groups were well sepa-

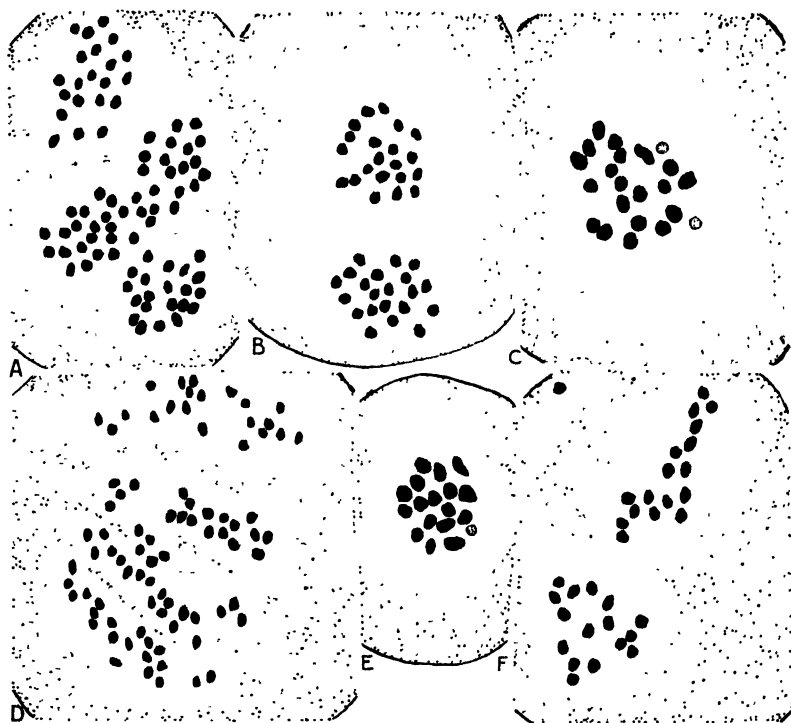


FIGURE 4. Chromosomes in the Mammoth and in Logan $\times$ Mammoth hybrids. A, Homotypic anaphase in the Mammoth, B, homotypic metaphase in the Cory, C, heterotypic prophase in F_1 Logan $\times$ Mammoth hybrid (no. 12), D, homotypic anaphase in a sister hybrid plant (no. 10), E and F, heterotypic and homotypic metaphase, respectively, in pentaploid F_1 Logan $\times$ Mammoth hybrids (no. 7 and no. 4)

rated. At this stage there is no longer the difficulty of distinguishing between univalent and bivalent chromosomes.

Two plants from this group of hybrids were found to be pentaploid. These plants showed many irregularities in their chromosome distribution during the meiotic phases. Figure 4, E, is a typical heterotypic metaphase from plant 7, showing both univalent and bivalent chromosomes on the spindle. Figure 4, F, is a homotypic metaphase from plant 4, showing two major chromosome groups and an extended chromosome near the periphery of the cell.

The character of the pollen of these eight F_1 hybrids is shown in table 2. The percentage of sterility ranges from that of almost completely sterile plants to that of largely fertile plants. The two pentaploid plants were sterile, but the amount of aborted pollen in some of the hexaploid plants was large also, indicating that the incom-

patibility of the chromosomes from the two parents is sufficient in some plants to lead to the abortion of an appreciable amount of pollen, although the chromosome number is an even multiple of the basic number 7.

LOGAN $\times$ YOUNG HYBRIDS

The Young, which originated as a cross of the Austin Mayes $\times$ Phenomenal (very similar to the Logan) in Louisiana, is a plant about as vigorous as the Logan. Its canes are slenderer and are even longer. Its fruit is large but intermediate in color between that of the Logan and that of the Austin Mayes.

Of 169 crosses of Logan $\times$ Young, 21 set half or more of their drupelets; the remainder were entirely or nearly sterile. No contrasting characters were apparent. Table 2 shows that in the nine hexaploid Logan $\times$ Young hybrids the amount of aborted pollen varied. In a few plants the amount was large, indicating that some of the chromosomes from the two parents were incompatible. The

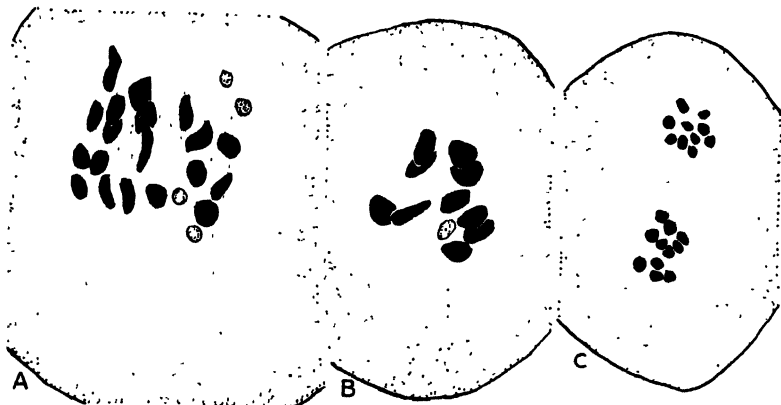


FIGURE 5.—Chromosomes in F_1 Logan $\times$ Young hybrids A, Heterotypic prophase in plant no. 1; B, heterotypic prophase in triploid plant no. 7, C, homotypic metaphase in plant no. 7

triploid plant was almost completely sterile, a condition usually found in triploid *Rubus*.

The Young likewise has 21 as its haploid number. Ten F_1 hybrids of the Logan $\times$ Young cross were studied cytologically. Of these, nine had 21 as their haploid chromosome number. The behavior of the chromosomes of these nine hexaploid hybrids during the reduction phases was less regular than that of hexaploid Logan seedlings, but more regular than that of some of the Logan $\times$ Mammoth hybrids.

Figure 5, A, shows a heterotypic prophase from plant no. 1. Four chromosomes are unpaired in this figure. At this phase unpaired chromosomes were frequently seen, but in many preparations the chromosomes were all paired and their distribution regular.

In plant 7 the chromosomes were exceptional in number and behavior. This plant had only $21\frac{1}{2}$ as its haploid chromosome number. The number of bivalents at diakinesis was variable, although most frequently there were 10 and a single univalent. Figure 5, B, shows a characteristic heterotypic prophase from the triploid plant.

At figure 5, C, is a homotypic metaphase showing 10 chromosomes on 1 plate and 11 on the other.

LOGAN $\times$ LUCRETIA HYBRIDS

The Lucretia, a horticultural variety of the eastern dewberry, likewise has 21 as its haploid chromosome number. Figure 6, A, shows 21 chromosomes on each plate of the homotypic metaphase from this variety. Of 19 crosses of the Logan $\times$ Lucretia 18 were sterile or nearly so, while 1 set a fair number of drupelets. Table 2 shows that the pollen condition of these crosses ranged from very little sterility to about 50 percent abortive pollen in the more sterile plants. The chromosome behavior and the large amount of sterile pollen in

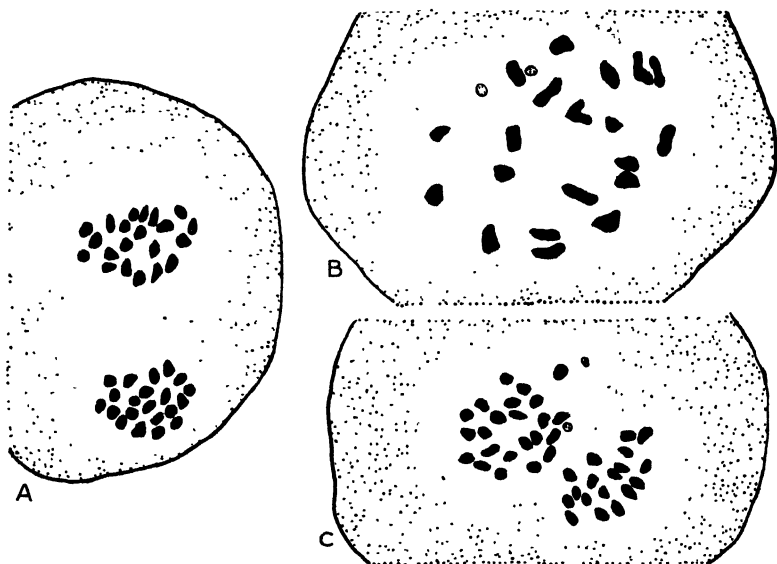


FIGURE 6.—Chromosomes in the Lucretia and in Logan $\times$ Lucretia hybrids. A, Homotypic metaphase in the Lucretia, B, diakinesis in Logan $\times$ Lucretia plant (no. 6), C, homotypic metaphase in Logan $\times$ Lucretia plant (no. 12).

some plants indicate that the chromosome complement of these plants is incompatible.

Nine F_1 Logan $\times$ Lucretia hybrids were studied and all were found to be hexaploid. The chromosomes of these few representative plants were variable in their behavior during meiosis. In some plants many irregularities in chromosome pairing were observed, whereas in others it was unusual to find univalent chromosomes in the early heterotypic phases. Figure 6, B, shows a late diakinesis from plant 6, one of the more fertile plants. Figure 6, C, shows a homotypic metaphase from plant 12, one of the more sterile plants.

RUBUS MACROPETALUS $\times$ LOGAN HYBRIDS

Several plants that were said to have resulted from a cross of selections from the wild *Rubus macropetalus* and the Logan were observed in the garden of J. W. Ware in western Washington. The plants had

large canes and handsome wine-colored fruit. The color of the fruit was the same on all the plants, although the size ranged from about that of the Logan to somewhat smaller. The flavor of one sort was better than that of any wild berries tested.

Several seedlings, supposedly of this cross, also were seen at Cheshire, Oreg. One was male, several were pistillate, and several were perfect-flowered and had fruit of the same color as the Ware plants.

Still another berry, supposedly a cross of *Rubus macropetalus* × Logan, appeared as a seedling in a garden near Florence, Oreg. It likewise had wine-colored fruit nearly as large as the Logan and of very fine flavor. This cross, as well as those of Ware, was perfect-flowered.

HIMALAYA × LOGAN HYBRIDS

A few seedlings, all very much alike and said to be a cross of the Himalaya and the Logan, were seen at Ware's ranch. The plants were young but bore a few well-developed berries nearly black in color, of high flavor, and nearly as large as those of the Logan.

CHROMOSOMES IN OTHER WESTERN RUBUS FORMS

With a view to a better understanding of breeding problems and of the origin and evolution of the western species of *Rubus*, the chromosome numbers of other western species were obtained. The Himalaya, as previously determined (1), was found to have 14 as the haploid chromosome number; *Rubus spectabilis* Pursh, the salmon-berry, 7; *R. parviflorus* Nutt., the thimbleberry, 7; and *R. leucodermis* Dougl., the western black raspberry, 7. The Lloyd George red raspberry has 7; the Cuthbert, 7, the Burbank Thornless (*R. inermis*, Willd., of Europe), 7; the Evergreen, 14; the Brainerd (Himalaya × an eastern blackberry), 21/2; and the Austin Thornless dewberry, 28.

DISCUSSION

In this western group of related forms of *Rubus* the haploid chromosome number ranges from 21 to 42. The members of the western group are distinct from other known groups of raspberries and blackberries in being dioecious. Dioeciousness has been found in only one other *Rubus* species, namely, *R. chamaemorus* L. (9), which has 28 chromosomes. It is interesting that dioecious species of *Rubus*, like dioecious species of *Fragaria*, occur only in forms with high chromosome numbers.

The haploid chromosome number (21) that characterizes the Logan is quite frequently found in *Rubus* species of the dewberry type. The behavior of the chromosomes of the Logan when associated with other *Rubus* varieties indicates that there is an appreciable amount of incompatibility between its chromosomes and those of the other varieties studied. In extreme cases this incompatibility leads to the production of very little viable pollen. No fully fertile crosses were obtained with the Young, the Lucretia, or even with the Mammoth, which like the Logan originated from a seedling of the Aughinbaugh and had 21 chromosomes. On the other hand, the supposed *Rubus macropetalus* × Logan crosses were fruitful.

Chromosome number is often indicative of the closeness of relationship of two species, but it seemed that a study of the behavior of the two chromosome sets when brought together in a hybrid plant might give a more satisfactory idea of the relationship of the two parent forms.

The chromosomes of the Logan show irregularities during meioses too infrequently to support the view that they are made up of two sets derived from *Rubus ursinus* and a red raspberry. In fact their behavior is no more irregular than that frequently found in a pure species.

If the Logan were of hybrid ancestry, with *Rubus macropetalus* as one parent, obviously its diploid number should exceed the haploid number of *R. macropetalus*. The haploid chromosome number of *R. macropetalus* is known only from the few collections made for this study. Neither 42, found in dioecious forms, nor 28, found in the Ideal Wild variety, are numbers that explain readily the presence of 42 somatic chromosomes in the Logan. It seems unnecessary to assume from either its chromosome number or its chromosome behavior that the Logan originated as a hybrid. From these considerations it seems probable that the Logan originated as a red-fruited perfect-flowered sport of the species herein designated as *R. loganobaccus*.

The discovery in the group of Logan. Young hybrids of one plant having the same chromosome number as the female parent suggests the possibility that *Rubus loganobaccus* is a haploid species derived from *R. ursinus* and *R. macropetalus*. However, it may be a survivor of former types that were pushed southward in glacial times.

Forms such as those collected in the wild and the Ideal Wild variety, having chromosome numbers intermediate between *R. loganobaccus* and *R. macropetalus*, may represent (1) remnants of *Rubus* species almost extinct, (2) hybrids between the three species discussed in this paper, or (3) aberrant forms of these species.

The excellent quality and fertility of the *Rubus macropetalus* Logan crosses indicate the possibility of using for breeding purposes selected forms of all three species of western trailing blackberries.

SUMMARY

A characterization is given of the wild trailing blackberries, or dewberries, of the Pacific coast (*Rubus loganobaccus*, *R. ursinus*, and *R. macropetalus*), and a study of their chromosomes is made with reference to the origin and relationships of the cultivated varieties, particularly the Logan. A new variety of *R. ursinus* (var. *monophyllus*) is described.

Rubus macropetalus has 42 chromosomes as its haploid number; *R. loganobaccus*, 21. Plants taxonomically similar to *R. loganobaccus* were found to have 28 or 35 chromosomes. The cultivated variety Ideal Wild, thought to have originated as a cross between *R. macropetalus* and the Logan, has 28 chromosomes.

The history and cytology of the Logan variety are discussed. The chromosome number of the Logan is 21; of 11 seedlings, 1 had 35/2 and the other 10 had 21 chromosomes.

Breeding experiments with the Logan gave the following results: The F_1 seedlings of the selfed Logan showed characteristics of the

Logan and not of raspberry. Of the seedlings selected, some matured earlier and some later than the Logan; the fruit of some of these selections was sweeter than that of the Logan.

Crosses of the Logan (21 chromosomes) $\times$ Mammoth (21) gave 162 plants that were nearly or entirely sterile and 2 that were partially fertile. Of 8 plants examined, 6 had 21 chromosomes and 2 had 35/2 chromosomes.

Crosses of Logan $\times$ Young (21 chromosomes) gave 148 plants that were nearly or entirely sterile and 21 that were partially fertile. Of 10 plants examined 9 had 21 chromosomes and 1 had 21/2 chromosomes.

Crosses of Logan $\times$ Lucretia (21 chromosomes) gave 18 plants that were nearly or entirely sterile and 1 that was partially fertile. Each of 9 plants examined had 21 chromosomes.

Plants believed to be crosses between *Rubus macropetalus* (42 chromosomes) and the Logan (21) gave some perfect-flowered fertile seedlings.

Other western species (*Rubus spectabilis*, *R. parviflorus*, and *R. leucodermis*) have 7 chromosomes each.

From the data presented herein it is concluded that the Logan probably originated as a red-fruited perfect-flowered sport of *Rubus loganobaccus*.

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EFFECT OF THE CALCIUM-PHOSPHORUS RELATIONSHIP OF THE RATION ON GROWTH AND BONE FORMATION IN THE PIG¹

By R. M. BETHKE, *associate (nutrition)*, B. H. EDGINGTON, *associate in animal pathology*, and C. H. KICK, *assistant in nutrition*, Department of Animal Industry, Ohio Agricultural Experiment Station

INTRODUCTION

Many studies have been made on the need of calcium, phosphorus, and vitamin D in the nutrition of the pig. The majority of these investigations dealt with the effect of mineral and vitamin deficient rations and how the deficiencies could be corrected. No specific information, so far as the writers are aware, is available regarding the effect of the calcium-phosphorus ratio of the ration on its relation to vitamin D on growth and bone formation in the pig. A study was therefore made of this phase of the subject, and the results are reported in the present paper.

REVIEW OF LITERATURE

McCollum and his associates (6, 9)² and Sherman and Pappenheimer (8) pointed out independently that the quantitative relation between the calcium and phosphorus in the food supply was, within certain limits of concentration, of great importance in determining whether an animal, like the rat, developed normal or pathological bones. Bethke, Steenbock, and Nelson (3) reported that the amount of antirachitic factor required by rats on a synthetic ration containing 0.655 percent of phosphorus varied inversely with the calcium content of the ration. This indicates the existence of a quantitative relation between vitamin D and calcium in effecting the utilization of the latter. More recent investigations at this station (2) revealed that the amount of vitamin D required by the rat is directly correlated with the calcium and phosphorus content of the ration and the ratio in which these elements are present. Evidence was also obtained which showed that increased levels or concentrations of calcium and phosphorus in the ration exerted beneficial effects on growth and on bone ash, but the effects were not so great as those exerted by the ratio of calcium to phosphorus. Similar observations (1) have been made with the chick. Haag and Palmer (5) also showed the importance of a more or less balanced condition of calcium, phosphorus, and magnesium salts in the ration for good growth and mineral retention in the rat. The same investigators also called attention to the importance of the vitamin-mineral interrelationship in the nutrition of the rat.

The importance of ample quantities of calcium and phosphorus in the ration of the pig has been shown many times. Maynard, Goldberg, and Miller (7) reported that even when calcium and phosphorus were in abundance in the ration of the pig, poor bone development

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² Reference is made by number (italic) to Literature Cited, p. 338.

frequently resulted which could be corrected by supplying vitamin D or its equivalent. Bohstedt et al. (4), at this station, also observed that under certain conditions improperly calcified bones occurred, even though the ration in question was supplemented with what was supposed to be ample quantities of calcium. Observations of practical farm feeding have indicated that large amounts of calcium in the form of calcium carbonate or ground limestone in the grain rations of pigs often produce unfavorable results.

EXPERIMENTAL PROCEDURE

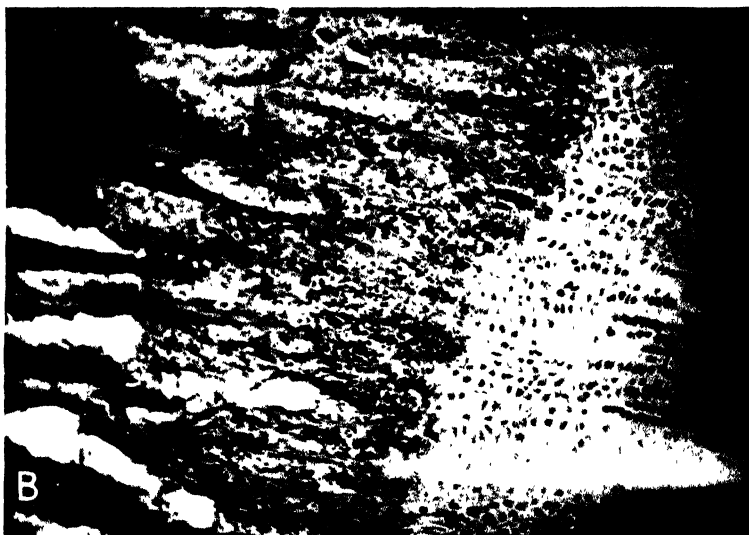
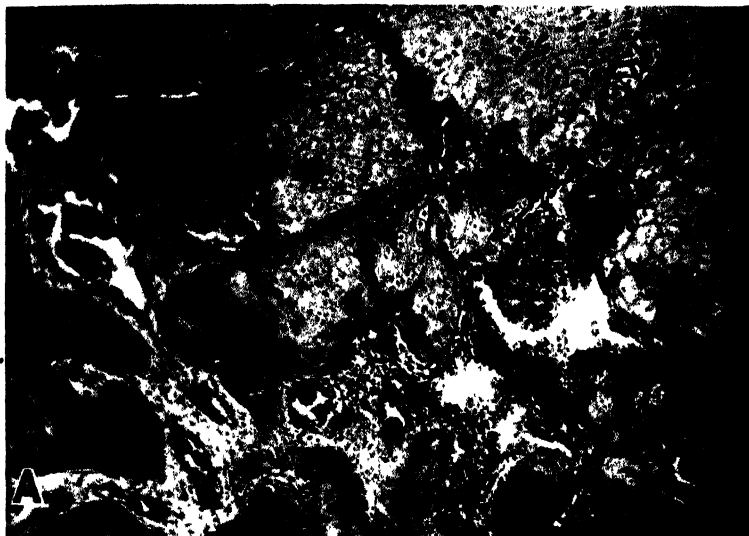
The findings and observations of these workers led the writers to investigate the importance of the calcium-phosphorus ratio in the nutrition of the rat and the pig. For experimental reasons, it was deemed advisable to use identical rations with the two species. A ration was desired that could be relied upon to produce incipient rickets in the rat in 3 to 5 weeks and that would also constitute a comparatively practical ration for the pig. After considerable preliminary investigation, a basal ration of 79.5 parts yellow corn, 20.0 parts soybean meal, and 0.5 part sodium chloride was adopted. This ration, when properly supplemented with minerals, had given good results in other studies with pigs. The same basal ration was used in the different experiments. The present report includes only the results of three experiments with pigs.

Five lots of six pigs each were used in the first experiment. The pigs were started on experiment shortly after weaning and were from 20 to 35 pounds in weight. All lots were housed in a central building provided with concrete floors. One lot had access to an outdoor paved runway, on which they were fed. A second lot had access to an outdoor dirt paddock but, like the other three lots, were fed indoors. The pigs were self-fed their respective rations for a period of 19 weeks, starting the latter part of June. At the end of the experiment all animals were slaughtered and given a routine post-mortem examination. Portions of ribs, including the costochondral junctions and both femurs, were removed from each pig for histological study and ash analysis. The average growth of the different lots is shown in figure 1. Table 1 shows the average daily feed consumption and the average breaking strength and ash analysis of the femurs. The percentage of ash is expressed on a moisture-free and fat-free basis.

TABLE 1.—*Effect of the calcium-phosphorus ratio of the ration on calcification in the pig*

Lot no	Additions to basal ration	Calcium and phosphorus in ration	Calcium-phosphorus ratio in ration	Average daily feed	Average weight of femurs	Average breaking strength	Average ash in femurs
				Pounds	Grams	Pound	Percent
a 1	None.....	Ca, 0.06, P, 0.33.	0.18	1.52	58.7	171±13	49.5±0.72
2	3 parts calcium carbonate.....	Ca, 1.16, P, 0.32	3.62	1.88	58.0	143±23	46.0±.63
3	3 parts calcium carbonate, 3 parts disodium phosphate.....	Ca, 1.14, P, 0.62.	1.84	3.08	111.7	799±51	61.4±.30
4	1 part ground limestone, 1 part bone meal (outdoor dirt runway).....	Ca, 0.62, P, 0.44	1.41	3.09	124.9	791±30	61.1±.33
5	3 parts calcium carbonate (outdoor paved runway)	Ca, 1.16, P, 0.32.	3.62	2.77	80.5	321±38	52.5±.43

* 1 pig dead in lot; averages based on 5 animals



PHOTOMICROGRAPHS OF THE COSTOCHONDRAL JUNCTION OF PIGS

A, No. 24, lot 2, experiment 1, showing distorted calcification resembling rickets. B, No. 8, lot 3, experiment 1, showing normal calcification.

The pigs on the basal ration (lot 1) exhibited marked evidence of a mineral deficiency, in the form of a stiffened, staggering gait. Several individuals in this lot were so severely affected that they were unable to walk. One of these died 2 days prior to the termination of the experiment. Another pig in this lot died early in the experiment from a ruptured bladder. Two showed signs of tetany: undoubtedly due to a low calcium intake. The addition of 3 parts of calcium carbonate

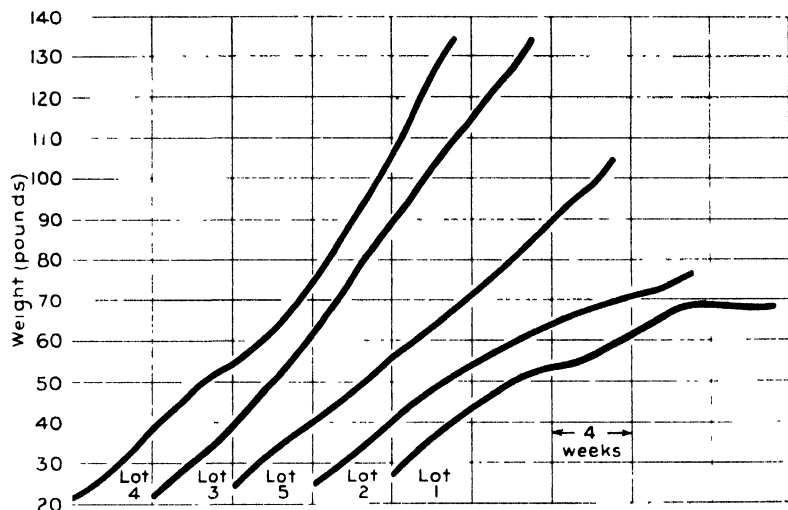


FIGURE 1. Average growth of different lots of pigs in experiment 1. Lot 1, basal ration only, lot 2, basal ration plus 3 parts calcium carbonate, lot 3, basal ration plus 3 parts calcium carbonate and 3 parts disodium phosphate, lot 4, basal ration plus 1 part ground limestone and 1 part bone meal (outdoor dirt runway), lot 5, basal ration plus 3 parts calcium carbonate (outdoor paved runway).

to the basal ration (lot 2), while causing an approximate twentyfold increase in calcium intake, did not improve the ration for growth or bone formation. All the pigs in this lot showed marked clinical symptoms of rickets, the diagnosis being further supported by the ash determinations of the femurs and the histological examination of the costochondral junctions (pl. 1, A). Changing the calcium-phosphorus ratio of the ration from 3.62 (lot 2) to 1.84 (lot 3) by the addition of 3 parts disodium phosphate resulted in increased feed intake, improved growth, and a normal breaking strength and ash content of the femurs. The costochondral junctions of the pigs from lots 3 and 4 also showed normal calcification (pl. 1, B). The beneficial effects of direct sunlight on growth and calcification are revealed in the results in lot 5. However, it is apparent that the physiological rays were not sufficient entirely to counteract the faults of the wide calcium-phosphorus ratio (3.62) and produce bones having a normal ash value and histological structure.

The results of the first experiment indicated that the calcium-phosphorus ratio of the ration exerted an effect on feed consumption and on bone calcification in the pig. To confirm the data already presented, and to obtain additional knowledge with respect to the effect of the calcium-phosphorus ratio on bone formation, a second experiment was initiated. The basal ration was the same as that used

in the first experiment but was supplemented with calcium and phosphorus in such quantities that the pigs in each lot received a ration possessing a different proportion of calcium to phosphorus. The trial was conducted during the winter months and involved 9 lots of 6 pigs each. These pigs weighed from 20 to 35 pounds at the beginning of the test. All lots were confined indoors and self-fed their respective rations. Straw was used for bedding. The experiment was continued for 18 weeks, when the animals were slaughtered and examinations made as in the previous trial. The rations and the results of the bone studies are presented in table 2. The average growth of the different lots is shown in figure 2.

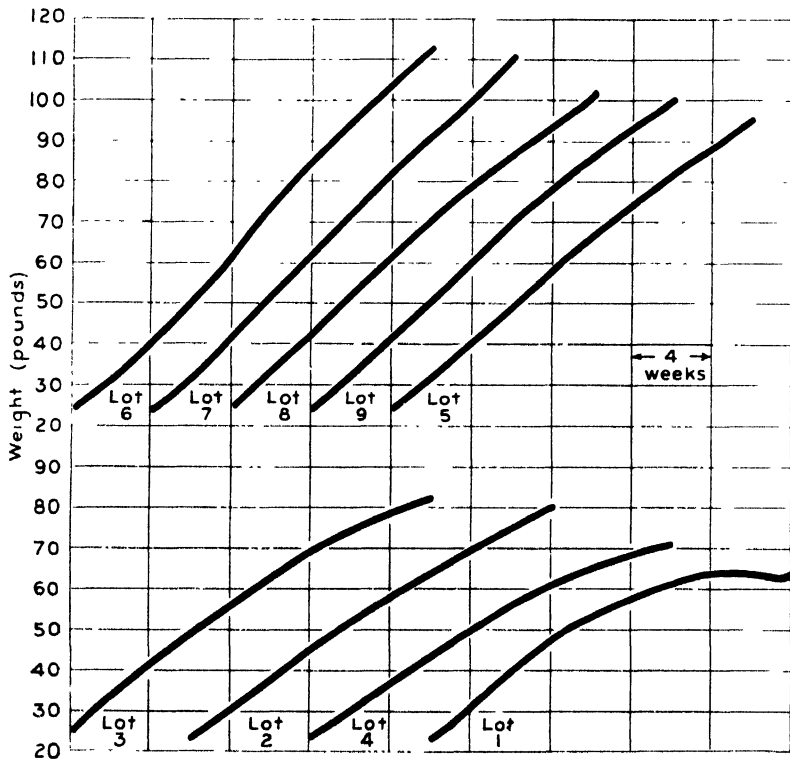


FIGURE 2.—Average growth of different lots of pigs in experiment 2. Lot 1, basal ration only; lot 2, basal ration plus 1 part calcium carbonate; lot 3, basal ration plus 2 parts calcium carbonate; lot 4, basal ration plus 3 parts calcium carbonate; lot 5, basal ration plus 3 parts calcium carbonate and 1 part disodium phosphate; lot 6, basal ration plus 3 parts calcium carbonate and 2 parts disodium phosphate; lot 7, basal ration plus 3 parts calcium carbonate and 3 parts disodium phosphate; lot 8, basal ration plus 2 parts calcium carbonate and 3 parts disodium phosphate; lot 9, basal ration plus 2 parts tricalcium phosphate.

The pigs on the basal ration, or the basal ration supplemented with varying amounts of calcium carbonate (lots 1, 2, 3, and 4) showed extreme symptoms of mineral deficiency. All the pigs in these lots, with the exception of three individuals in lot 3, were so severely affected that they were unable to walk. This necessitated placing their feed and water before them in small troughs. The pigs in the other lots (lots 5, 6, 7, 8, and 9), which received the basal

ration supplemented with different amounts of calcium and phosphorus, did not exhibit such extreme symptoms of a deficiency, although the individual pigs in these lots all showed varying degrees of stiffness in the latter part of the experiment.

TABLE 2.—*The effect of the calcium and phosphorus content of the ration on bone formation of pigs*

Lot no.	Additions to basal ration	Calcium and phosphorus in ration	Calcium-phosphorus ratio in ration	Average daily feed	Average weight of femurs	Average breaking strength	Average ash in femurs
		<i>Percent</i>		<i>Pound</i>	<i>Gram</i>	<i>Pound</i>	<i>Percent</i>
1	None.....	Ca, 0.06, P, 0.33	0.18	1.35	42.2	124±21	44.9±0.71
2	1 part CaCO ₃	Ca, 0.42, P, 0.43	1.27	1.90	52.2	143±12	46.3±.46
3	2 parts CaCO ₃	Ca, 0.79; P, 0.32	2.47	1.95	51.5	173±15	48.2±.57
4	3 parts CaCO ₃	Ca, 1.16; P, 0.32	3.62	1.55	48.2	149±18	46.9±.60
5	3 parts CaCO ₃ ; 1 part Na ₂ HPO ₄ .12 H ₂ O.....	Ca, 1.16, P, 0.43	2.70	2.40	67.5	287±26	54.0±.91
6	3 parts CaCO ₃ ; 2 parts Na ₂ HPO ₄ .12 H ₂ O.....	Ca, 1.15, P, 0.54	2.13	2.70	82.0	573±59	58.2±.57
7	3 parts CaCO ₃ ; 3 parts Na ₂ HPO ₄ .12 H ₂ O.....	Ca, 1.14, P, 0.64	1.78	2.72	91.5	493±53	55.6±.73
8	2 parts CaCO ₃ ; 3 parts Na ₂ HPO ₄ .12 H ₂ O.....	Ca, 0.78, P, 0.64	1.22	2.52	72.1	423±42	55.1±.45
9	2 parts Ca ₃ (PO ₄) ₂	Ca, 0.83, P, 0.73	1.14	2.41	71.5	384±37	55.9±.50

The results of the bone studies (table 2) show that the ratio of calcium to phosphorus and the amounts of these elements in the ration had a marked effect on the breaking strength and the percentage of ash in the femurs. The addition of 2 parts of calcium carbonate, or a change in the calcium-phosphorus ratio from 0.18 to 2.47 caused a significant increase in the percentage of ash, whereas further additions of calcium carbonate, with a widening of the calcium-phosphorus ratio to 3.62, decreased the ash content and breaking strength. Although the decreases in ash content and breaking strength are not statistically significant, they indicate that the ration became more rachitic upon the further addition of calcium and the widening of the calcium-phosphorus ratio. The inclusion of both phosphorus and calcium in the ration caused a significant increase in ash content and in the breaking strength of the femurs. It is apparent that phosphorus, as well as calcium, and the proportion in which they are present in the ration may serve as limiting factors in calcification processes. The data also suggest that the requirements of the pig for vitamin D can be minimized by properly adjusting the calcium and phosphorus content of the ration. Although no vitamin D fed lots were included in the trial, it is obvious that a ration which causes bones to have an ash content between 55 to 60 percent will not require so much of the antirachitic factor for normal calcification as will one that causes bones to have 45 to 55 percent of ash. The results of the histological examinations of the costochondral junctions confirmed the bone-ash data.

It is of interest to note that, in general, the ash values of the second experiment are lower than those of the first. The writers are of the opinion that this difference is primarily due to vitamin D storage and control. The pigs used in the last test were farrowed in October and started on test in December; whereas those in the first experiment were farrowed in April and had access to May and June sunshine before they were started on experiment the latter part of June.

The results of the two previous experiments conclusively showed that bone formation in the pig was influenced by the calcium-phosphorus ratio of the ration. It still remained to be determined whether the level or concentration of calcium and phosphorus at different ratios would exert an effect on bone formation. Accordingly, a third experiment, comprising 10 lots of 6 pigs each, was started. The pigs used were farrowed in January and had access to the outdoors prior to the start of the experiment in April. They were treated for worms and immunized against cholera before being placed on test. All lots were confined indoors in concrete paved pens and self-fed their respective rations. The experiment ran for 112 days, when the animals were killed for post-mortem examination. Femurs and costochondral junctions were saved for examination, as in the previous experiments. Two days prior to the termination of the experiment, the blood of the individual pigs was analyzed for calcium by the Clark and Collip procedure and for inorganic phosphorus by the method of Briggs. The blood was obtained by tail bleeding and allowed to refrigerate overnight before the serum was drawn off for analysis.

The yellow corn-soybean meal ration was supplemented with such amounts of calcium carbonate and steamed bone meal as to obtain approximately the same three calcium-phosphorus ratios at three different concentrations. In order to have a vitamin D fed lot for comparison, the same ration as fed to lot 5 was supplemented with 0.5 part of aerated cod-liver oil and fed to lot 10. The aerated cod-liver oil was potent in vitamin D but practically devoid of vitamin A.

TABLE 3—*Effect of a varying calcium and phosphorus intake on growth, feed utilization, blood composition, and bone formation in the pig*

Lot no	Additions to ration		Calcium in ration	Phosphorus in ration	Calcium-phosphorus ratio	Average initial weight of animals	Average gain in weight	Average daily gain
	CaCO ₃	Steamed bonemeal						
	Parts	Parts	Percent	Percent		Pounds	Pounds	Pounds
1	0.7	---	0.33	0.33	1.0	37.2	109.5	0.98±0.050
2	1.6	---	.64	.42	2.0	37.5	112.8	1.01±.034
3	2.6	---	1.05	.31	3.4	38.8	77.1	.69±.062
4 ^a	2	1	.47	.46	1.0	35.2	126.0	1.16±.025
5	1.5	1	.92	.45	2.0	34.7	131.8	1.18±.030
6	2.8	1	1.36	.45	3.0	37.3	116.3	1.01±.038
7	---	2	.72	.60	1.2	37.8	142.8	1.27±.033
8	1.3	2	1.16	.58	2.0	39.0	142.1	1.27±.029
9	3.1	2	1.75	.57	3.0	33.0	144.5	1.29±.048
10 ^b	1.5	1	.92	.45	2.0	36.2	159.0	1.12±.045

Lot no.	Average daily feed	Feed intake per 100 pounds of gain	Blood analysis ^c		Average weight of femurs	Average breaking strength	Average ash in femurs	Degree of calcification ^d
			Ca in serum	P in serum				
	Pounds	Pounds	Mil.-tigrams	Mil.-tigrams	Grams	Pounds	Percent	
1	3.22	329.1	10.6	4.4	106	439±38	57.7±0.70	14
2	3.61	358.8	11.4	4.4	104	433±18	57.3±.66	15
3	2.50	364.8	11.1	4.9	96	351±37	55.4±.63	10
4	3.82	330.3	11.0	6.6	137	632±33	60.6±.59	20
5	3.94	331.9	11.6	6.5	133	801±51	63.5±.27	23
6	3.72	358.0	11.3	5.8	128	708±26	60.1±.48	22
7	4.88	343.5	10.3	6.3	151	785±5	62.8±.43	21
8	4.47	352.4	10.4	6.4	152	961±40	62.1±.52	24
9	4.30	333.6	11.5	6.6	142	808±29	62.3±.07	22
10 ^b	4.38	309.0	11.4	6.4	163	812±16	62.4±.28	24

^a 1 pig died after being on experiment 13 weeks, from fracture of both ilia.

^b 0.5 part of aerated cod-liver oil included in ration.

^c Expressed in mg per 100 cc of serum.

^d A score of 24 indicates normal calcification, and in descending order abnormality.

The growth and feed records are presented in table 3. It is evident that by using a ration having a calcium-phosphorus ratio of 3.0, with a phosphorus content of 0.31 or 0.45 percent, the rate of gain is significantly decreased and the feed utilization per unit of gain slightly increased over the lower ratios. This, however, was not observed when the phosphorus was increased to approximately 0.60 percent. It appears from these observations that phosphorus as well as the calcium-phosphorus ratio was a limiting factor at the two lower concentrations. This is particularly apparent when the performance of lots 1, 2, and 3, which were fed a ration with a phosphorus content of about 0.31 percent, are compared with lots 4, 5, and 6, which had about 0.45 percent of phosphorus in their ration. The beneficial effects of acrated cod-liver oil on growth and feed utilization are revealed by comparing the results of lots 5 and 10. The writers are not certain that the increased gains and better feed utilization are entirely due to vitamin D. Other unpublished data suggest that some other factor in the cod-liver oil, aside from vitamin D, might have exerted a favorable influence.

The results of the blood and bone analysis, also presented in table 3, show that the inorganic phosphorus content of the blood serum, and the breaking strength and ash content of the femurs from the animals in lots 1, 2, and 3 were below normal. It is thought that these results indicate that phosphorus was a limiting factor, since an increase in the phosphorus content of the ration, irrespective of the amount of calcium present, resulted in a significant improvement. The blood and bone data, in this respect, corroborated the growth and feed-utilization results shown in table 3.

The effects of the calcium-phosphorus ratio on bone formation are not so apparent in this trial as in the previous experiments. However, the data in general support the previous observations that the calcium-phosphorus ratio, within certain limits of concentration, influences calcification. The experiment also shows that the concentration of calcium and phosphorus in the ration is a factor in growth and bone formation. When the percentage of these elements in the ration was increased, normal blood and bone composition resulted, irrespective of the ratio or the presence of added vitamin D. The data, in general, show that it is possible to so regulate the amount and proportion of calcium to phosphorus in the ration of the pig as to minimize the requirements for the antirachitic vitamin for normal growth and bone formation.

The results of the histological examinations (table 3) of the costochondral junctions substantiated the results of the ash analysis. For the sake of comparison, the results are expressed numerically. These figures were arrived at by arbitrarily assigning a certain score to the degree of calcification in each pig and then totaling the same for each lot. On this basis a score of 24 would indicate normal calcification throughout the lot, and in descending order an abnormal calcification, suggestive of rickets.

SUMMARY

The calcium-phosphorus ratio of the ration is a factor in growth and bone formation in the pig. In general, best results were obtained with a calcium-phosphorus ratio between 1.0 and 2.0. When the proportion of calcium to phosphorus was greater than 3.0, the pigs became more rachitic and the requirements for vitamin D were increased.

The concentration of calcium and phosphorus in the ration also exerted an effect on growth and bone formation.

Phosphorus, as well as calcium, may be a limiting factor in growth and calcification. Evidence was obtained indicating that the phosphorus content of the ration should not be less than approximately 0.60 percent for good growth and bone formation in the absence of added vitamin D.

The requirement of the pig for vitamin D can be minimized by properly adjusting the calcium and phosphorus content of the ration.

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PHYSIOLOGIC SPECIALIZATION OF *SPHACELOTHECA CRUENTA* (KÜHN) POTTER¹

By L. E. MELCHERS²

Head, Department of Botany, Kansas Agricultural Experiment Station

INTRODUCTION

The loose kernel smut, *Sphacelotheca cruenta* (Kühn) Potter, is occasionally found in small quantities in fields of sorghum, *Sorghum vulgare* Pers., although it is not widely distributed over the United States. An account of its discovery, its geographic distribution, and the morphologic characters of the causal organism have been adequately discussed (4, 6, 8).³ While *S. cruenta* is much less destructive in America than the fungus causing covered kernel smut of sorghum, *Sphacelotheca sorghi* (Link) Clinton, it is of scientific interest because it is known to hybridize with the latter (7, 8).

No extensive test to determine the pathogenicity of *Sphacelotheca cruenta* to different varieties of sorghum has been reported, although general observations on varietal response have been made by Reed (5). In 1918 Kulkarni (1) obtained seed of milo from the United States and inoculated it with spores of *S. sorghi* and *S. cruenta* from India. He reported that out of a total of 645 heads obtained, 3 were attacked by *S. sorghi* (0.47 percent infection) and 50 by *S. cruenta* (7.8 percent infection). Unquestionably Kulkarni's results for both *S. sorghi* and *S. cruenta* were authentic⁴ and in the light of present knowledge he undoubtedly was dealing with physiologic forms of the two species of sorghum kernel smut which differed from those common in the United States. At the time of his publication, however, specialization in the smut fungi had not been reported.

METHODS AND MATERIALS

In 1928 C. H. Ficke found a specimen of loose kernel smut in a field containing many varieties of sorghum. The discovery was of particular interest for two reasons: (1) But one head of loose kernel smut was found although there was an abundance of covered kernel smut in the field, and (2) the diseased head was that of a feterita plant, a sorghum resistant to practically all forms of *Sphacelotheca sorghi* and hitherto reported as highly resistant to attacks of *S. cruenta*. Martin and Ratliffe (2) recently reported some plants of feterita attacked by *S. cruenta*. In one other instance a feterita plant was reported to be attacked by the loose kernel smut (8), but no studies were made although the authors suspected that it might be explained on the basis of physiologic specialization.

In 1929, the smut collected by Ficke was increased so as to provide sufficient inoculum for varietal studies. In 1930 a specimen of

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³ Reference is made by number (italic) to Literature Cited, p. 342.

⁴ This smut, according to Reed, was originally obtained in 1921 or 1922 from S. L. Ajreker, Poona Agricultural College, Poona, India. The specimen obtained from Reed in 1930 was on Black Amber sorgo.

Sphacelotheca cruenta was obtained through the courtesy of G. M. Reed, of the Brooklyn Botanic Garden, N. Y.⁴ These two collections of the loose kernel smut were used in the varietal studies herein reported. The experiments were conducted in the field at Manhattan, Kans., during the years 1930-32, inclusive.

The smut reaction of 35 varieties of sorghum was studied. Not all of these varieties were grown in 1930 and 1931, however. Self-pollinated seed⁵ was used to insure purity of the variety. The same methods of inoculating seed, planting, and keeping field records were used as had previously been employed in the varietal studies with *Sphacelotheca sorghi* (3, 6). From 100 to 150 plants of each variety constituted a row. The relative susceptibility of the variety was determined from the number of smutted plants that appeared in the row.

EXPERIMENTAL RESULTS

The results of 3 years' work are presented in table 1. On the basis of resistance and susceptibility, the different varieties of sorghum have been arranged arbitrarily in four groups, A, B, C, and D. Environmental conditions in 1931 and 1932 were apparently not so favorable for infection as in 1930, for the percentages of infection were appreciably lower in practically all varieties.

It will be noted that two distinct physiologic forms of *Sphacelotheca cruenta* were found. The form obtained from India is designated form 1, and that discovered at Manhattan, form 2. Kafir $\times$ feterita K.B. 2686 and Pierce kaferita K.B. 2547 are resistant to form 2, whereas these varieties are readily attacked by form 1. On the other hand, form 2 attacks Red Amber $\times$ feterita K.B. 2570 and K.B. 2501, and White Yolo K.B. 2525 very readily, whereas form 1 infected but 1 plant out of nearly 1,000 inoculated.

In general, the milos, feteritas, hegari, and Dwarf Shantung kaoliang are extremely resistant to both forms; the studies thus far made indicate that they are immune.

The varieties in group B, which includes Dwarf Yellow milo, certain feteritas, feterita hybrids, Fargo Straightneck milo, Grohoma, and White durra, are only slightly susceptible to both forms of *Sphacelotheca cruenta*.

Group C, consisting of Premo, Red Amber, shallu, and Weskan, is highly susceptible to both forms of the organism.

Group D comprises a number of varieties which may be susceptible to one form and resistant to the other. Several varieties in this group, it will be noted, may be used as differential varieties in separating form 1 from form 2.

The results of these tests show conclusively that the loose kernel smut of sorghum obtained from India is distinct from that collected at Manhattan. At present it is not known whether there are additional physiologic forms of *Sphacelotheca cruenta*, but it seems very probable that other forms could be determined if new collections of the smut were available and the range of sorghum varieties extended.

In hybridization studies by Rodenhiser (?) in which he crossed *Sphacelotheca sorghi* with *S. cruenta*, the collections of *S. cruenta* which were supplied by the writer and referred to by Rodenhiser as C1 and C2, are here reported as physiologic forms 1 and 2.

⁴ See footnote 4, p. 339.

⁵ Members of the Department of Agronomy kindly furnished seed of some varieties.

TABLE 1.—Summary of reaction of sorghum varieties to 2 physiologic forms of *Sphacelotheca cruenta*, Manhattan, Kans., 1930-32

[Percentage of plants showing smut of form indicated]

Group and variety	K B ^a no	Form 1				Form 2			
		1930	1931	1932	Average	1930	1931	1932	Average
Group A:									
Spur feterita.....	2540	0	0	0	0	0	0	0	0
Dwarf Yellow milo, row no. 3, 1924.....	2515	0	0	0	0	0	0	0	0
Standard Yellow milo.....	2521	—	—	0	0	—	—	0	0
Standard White milo.....	2533	—	—	0	0	—	—	0	0
Dwarf Yellow milo × Pink.....	2693	—	0	0	0	—	0	0	0
Hegari selection.....	2537	0	0	0	0	0	0	0	0
Do.....	2518	0	0	0	0	0	0	0	0
Schrock selection.....	2511	—	—	0	0	—	—	0	0
Dwarf Shantung kaoliang, C.I. • 293.....	2565	—	—	0	0	—	—	0	0
Group B									
Dwarf Yellow milo, C.I. 332.....	2555	0	0	0	0	0	0	1 1	.4
Red Leaf feterita.....	2543	0	10	0	3 3	0	0	0	0
Feterita hybrid.....	2687	8 4	0	2 8	3 7	0	7	0	.2
Feterita, S. P. I. • 51989.....	2690	7 1	4 4	0	3 8	1 4	2	0	1 1
Fargo Straightneck milo.....	2677	2 3	0	0	8	0	0	1 1	1 4
Red Amber × feterita, row no. 56.....	2573	0	0	0	0	3 9	0	0	1 3
Red Amber × feterita, row no. 25.....	2562	0	0	0	0	15 6	0	0	5 2
Grohon.....	3032	—	5 1	8 6	6 9	—	3 3	1 5	2 4
White durra.....	2528	—	—	1 2	1 2	—	—	3 5	3 5
Group C									
Premo.....	2688	0	26 2	15 9	14 0	20 3	12 0	12 5	14 9
Red Amber.....	32313	—	—	15 6	15 6	—	—	16 0	16 0
Shallu.....	2542	90 2	31 0	33 6	51 6	52 6	18 5	29 5	33 5
Weskan.....	2522	—	—	45 3	45 3	—	—	46 2	46 2
Group D									
Early White milo.....	3026	—	—	10 5	10 5	—	—	0	0
Pierce kaferita.....	2547	59 4	10 4	0	23 3	0	1 4	0	.5
Kafir × feterita.....	2686	47 6	9 0	11 2	22 6	0	1 1	0	.4
Kafir × milo 26-3-1-1.....	2561	63 2	10 0	0	24 4	1 7	0	0	.6
Manchu Brown kaoliang.....	2568	41 8	16 0	2 5	21 1	6 9	2 6	4 6	4 7
Darso.....	3003	11 2	19 3	5 0	22 8	6 8	3 9	8 8	6 5
White darso.....	3002	43 8	49 6	9 4	31 3	7 6	10 0	12 0	9 9
Acme broomcorn.....	2558	88 0	12 5	8 2	36 2	20 0	8 3	4 0	10 8
Kansas Orange.....	3048	85 1	36 0	9 5	43 5	19 5	6 9	17 7	14 7
Blackhull.....	3025	50 8	23 6	16 6	30 3	29 9	5 7	10 7	15 4
Red Amber × feterita, row no. 57.....	2570	0	0	0	0	30 3	1 2	3 6	11 7
Red Amber × feterita, row no. 12.....	2501	0	0	0	0	47 5	3 1	8 9	19 8
White Yolo.....	2525	1 9	0	0	.6	21 7	14 1	5 2	13 6

^a K B, C. I., and S. P. I. indicate, respectively, accession numbers of the department of botany, Kansas Agricultural Experiment Station, Division of Cereal Crops and Diseases, and Division of Foreign Plant Introduction, U. S. Department of Agriculture.

It is apparent that *Sphacelotheca sorghi* and *S. cruenta* are equally virulent on some varieties of sorghum. A larger number of the so-called resistant sorghums are attacked by *S. sorghi*, however, because of the presence of five physiologic forms of that organism (3).

SUMMARY

Two physiologic forms of *Sphacelotheca cruenta* have been found, and these are designated forms 1 and 2. They may be separated on the basis of their pathogenicity to various sorghums.

Certain of the feteritas, milo, hegari, Schrock, and Dwarf Shantung kaoliang are highly resistant to both forms.

Form 1 attacks kafir × feterita and Pierce kaferita very readily, while form 2 does not infect these varieties to any extent. On the other hand, form 2 attacks Red Amber × feterita and White Yolo heavily, while these sorghums are immune from form 1.

Thirty-five varieties of sorghums were tested over a 3-year period, but not all were grown in 1930 and 1931. These studies indicate that

certain varieties of *feterita* and *milo* generally regarded as immune may be attacked by one or both forms of *S. cruenta*.

It is believed that other forms of *S. cruenta* may be found if additional collections of this smut are tested on a more extensive group of sorghums.

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BELATED DEVELOPMENT OF KERNEL SMUT (*SPHACELOTHECA SORGHII*) IN APPARENTLY HEALTHY SORGHUM PLANTS¹

By L. E. MELCHERS²

Head, Department of Botany, Kansas Agricultural Experiment Station

INTRODUCTION

One of the interesting features of infection experiments with kernel smut of sorghum, *Sphacelotheca sorghi* (Link) Clinton, is that the number of diseased plants seldom exceeds 50 percent even though heavily inoculated seed of highly susceptible varieties is planted under conditions favorable for the development of the disease. This peculiar characteristic has been noted repeatedly, and was indicated in a paper³ concerning the resistance shown by different varieties of sorghums to *S. sorghi* over a period of 6 years in four regions of the United States. It has been observed that these relatively low percentages of kernel-smut infection in susceptible sorghums under field conditions are not greatly increased by the variable environment of the field. These results are unlike those obtained in other cereal diseases, such as bunt in wheat and the smuts in oats, where infections close to 100 percent may be obtained.

Experiments were conducted to determine whether this lack of smut development was due to an escape from infection as suggested by Martin,⁴ or whether the disease merely failed to develop despite the presence of the smut organism in latent form in the host tissues. Studies were made of the response of different sorghum varieties (resistant and susceptible) to certain plant mutilations, and comparative tests were made with several physiologic forms of the kernel-smut fungus to learn whether all of them behave in a similar manner.

EXPERIMENTAL METHODS

The sorghum varietal plots which were used in studies on the physiologic forms of kernel smut⁵ served also for the experimental material in the studies here reported. Self-pollinated seed of the several varieties of sorghum was heavily inoculated with chlamydospores of several physiologic forms of *Sphacelotheca sorghi* and planted at a time favorable for maximum infection.

The inoculum for these tests was prepared in two ways: (1) A composite mass of chlamydospores from several different forms of the organism was used; and (2) the different physiologic forms were kept separate so that their characteristics with respect to latent

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² The writer gratefully acknowledges the assistance given by C. H. Ficke, C. O. Johnston, F. B. Bosley, and C. A. Wismer in these investigations.

³ REED, G. M., and MELCHERS, L. E. SORGHUM SMUTS AND VARIETAL RESISTANCE IN SORGHUMS. U.S. Dept. Agr. Bul. 1284, 66 p., illus. 1925.

⁴ MARTIN, J. H., and RATLIFF, G. T. LOOSE KERNEL SMUT ON FETERITA. (Phytopathological note) Phytopathology 17: 338-339. 1927.

⁵ MELCHERS, L. E., FICKE, C. H., and JOHNSTON, C. O. A STUDY OF THE PHYSIOLOGIC FORMS OF KERNEL SMUT (*SPHACELOTHECA SORGHII*) OF SORGHUM Jour. Agr. Research 44: 1-11, illus. 1932

smut invasion might be determined in both susceptible and resistant varieties of sorghum.

The details of inoculation, spacing, planting, etc., which were followed in these experiments are given in another publication.⁶ Each row contained from 100 to 150 plants of the variety, selection, or hybrid. Approximately 105 varieties, selections, and hybrids of sorghum, *Sorghum vulgare* Pers., were used during the 4 years in which these studies were made.⁷ Not all were planted each year, however, as table 1 shows.

It is a simple matter to determine whether a sorghum head is affected with kernel smut by the time the head emerges from the boot. If the head is normal, the stamens will soon extrude from the florets; but if kernel smut is present, the stamens do not appear, and a close examination will reveal the young smut galls or hypertrophied ovaries. It was at this stage that the apparently unsmutted plants were selected from each variety, selection, or hybrid for the experiments herein described.

Fifteen to twenty plants with unsmutted primary panicles were tagged in each row. These plants were mutilated in one of two ways during the course of the experiments. In 1929 and 1930 the normal primary heads were cut off at a point approximately 6 inches below the base of the panicle (fig. 1, A), and in 1931 and 1932 entire plants were also cut off at the level of the ground or a few inches above the ground (fig. 1, B).

Treatments of this type will cause the development of new shoots from axillary buds when the plant is merely beheaded and from sucker buds when the original stem is severed at the ground. The beheading treatment proved to be the better for this test, as heads were produced on the new growth in every instance, while the new tillers from sucker buds were seldom able to produce an inflorescence before frost. When the growing season was long enough for the new tillers to head out, the panicles in these tillers were smutted in several cases, indicating that there is mycelium of kernel smut within some plants of susceptible varieties which fail to produce smutted heads in the main tiller.

EXPERIMENTAL RESULTS

In Table 1 are shown (1) the percentages of kernel smut that occurred on the main heads of the varieties, selections, or hybrids as they grew naturally in the test plots, and (2) the percentages of kernel smut that developed on the same varieties, selections, and hybrids as a result of cutting back the apparently smut-free plants. The percentages of smut infection produced by cutting back were determined by dividing the number of heads that produced smut after this treatment by the number of mutilated plants. The percentages thus obtained should be a fair representation of the number of plants that are actually invaded by the kernel-smut fungus but that show no external indications of being diseased and so are usually counted as smut-free.

⁶ REED, G. M., and MELCHERS, L. E. Op. cit.

⁷ The writer is indebted to John H. Parker and A. F. Swanson, of the Kansas Agricultural Experiment Station and the Fort Hays Branch Experiment Station, respectively, for the seed of many of the varieties.



FIGURE 1.—A, Belated development of covered kernel smut of sorghum in axillary heads of Sagrain. The primary panicle was not smutted when it emerged from the boot. It was cut back as shown in the illustration. The plant, however, was infected with the smut organism as evidenced by the production of smutted axillary heads which developed later. B, Belated development of covered kernel smut of sorghum in secondary shoot or tiller of Kansas Orange $\times$ milo. The primary panicle was not smutted when it emerged from the boot. The entire plant was cut off near the ground. The new tiller produced a panicle that was affected by the kernel smut, *Sphacelotheca sorghi*.

TABLE 1.—Percentage of kernel smut in mutilated sorghum plants as compared with the percentage on primary heads of nonmutilated plants: Manhattan, Kans., 1929-32

Group, variety, or selection	Accession no ^b	Percentage of kernel smut on — ^a									
		Primary heads, plants not cut back				Axillary heads of cut- back plants ^c				Heads of secondary tillers of cut-back plants	
		1929	1930	1931	1932	1929	1930	1931	1932	1931	1932
Sorgo											
Atlas selection.....	K B 2505.....	21.4	3.3			0	0				
Atlas selection no. 95.....	K.B. 2877.....	26.2	41.0			0	0				
Atlas selection no. 100.....	K B. 2878.....	26.7				0					
Dwarf Sumac.....	K B. 2576.....	32.4	32.6			7.5	13.3				
Standard Sumac.....	K B 2902.....	27.4	27.9			0	0				
Red Amber.....	K B 2504.....	16.2	2.0			0	0				
Leoti Red.....	F.C.I. 6610.....	31.3	7.4			55.0	40.0				
Kansas Orange.....	K B. 2560.....	25.6	20.7			10.0	13.3				
Japanese Honey Drip.....	K B. 2876.....	29.6	26.0			0	6.7				
Honey.....	K B 2874.....	32.6									
Weskan.....	K B 2522.....	26.1	11.6			10	13.3				
Freed.....	K B. 2519.....	20.8	1.7			0	0				
Dwarf Freed.....	K B 27107.....	32.4	0			0	0				
Pink Freed (Tribune) no.....	101-5 (1926).....	35.2	33.0			0	0				
Kansas Orange.....	K B 3048.....			10.2	9.0			5	0	0	5
Red Amber X feterita:											
	(K B 2552.....	0	2.0			0	0				
	K B. 2513.....	0	0			0	0				
	K B 2509.....	3.0	0			0	0				
Selection.....	K B 2562.....	0	0	0	0	0	0	0	0	0	0
	K B 2501.....	0	2.1	2.1	1	0	0	0	0	0	0
	K B 2567.....		1.6			0	0				
	K B 2573.....	0	0	0	0	0	0	0	0	0	0
	K B 2570.....	21.5	6.0	0	5.5	25	13.3	0	0	0	0
Feterita:											
Selection.....	K B. 2503.....	0	0			0	0				
Hays selection.....	C.I. 182-1.....	0.4	0			0	0				
Dwarf feterita no. 6.....	K B. 2820.....	3.7	1.2	0	0	0	0	0	0	0	0
Red Leaf.....	K B. 2543.....	2.2	2.8	0	0	0	0	0	0	0	0
Do.....	K B 2544.....	2.5	2.5			0	0				
Feterita.....	S.P.I. 51989.....	0.5	2.1	0	0	0	0	0	0	0	0
Spur.....	K B 2540.....	0	0	0	0	0	0	0	0	0	0
Kafir:											
Reed.....	C.I. 628.....	3.8	20.8			10	0				
Western Blackhull.....	K B. 27102.....	21.0	29.4			0	0				
Dawn.....	K B 2695.....	19.5	25.0			0	0				
Do.....	K B 2538.....	20.6	20.0			0	0				
Bishop.....	C.I. 814.....			0	0			0	0	0	0
Pink.....	K B. 2546.....	26.7	19.7			0	0				
Do.....	K B 2506.....	28.5	13.3			0	0				
Do.....	F.C.I. 9091.....	35.6	18.8			5	13.3				
Early Pink.....	K B 2824.....	27.2	19.7			5	20.0				
Blackhull.....	K B. 2535.....	16.2	13.9			5	26.7				
Do.....	K B 3047.....			17.4	15.1			0	0	0	0
Red kafir.....	K B 2545.....	25.2	28.2			15	33.3				
Club.....	C.I. 901.....	20.4	8.0			0	0				
Modoc Pink Freed.....	K B. 2831.....	20.1	30.0			0	0				
Sunrise.....	K B 2523.....	21.1				0					
Kafir X feterita:											
Hybrid.....	F.C.I. 8920.....	2.4	1.8			0	0				
Pierce kafirita.....	K B. 27101.....	8.4	1.2			0	0				
Pierce kafirita selection.....	K B. 2549.....	32.9	28.2			5.0	6.7				
Do.....	K B. 2547.....	4.1	0	0	0	0	6.7	5	0	0	0
Kafirita.....	C.I. 812.....			7.6	7.6			0	0	5	(^d)
Hybrid.....	K B. 2686.....	41.9		0	1.6	5.0		0	0	0	0
Premo.....	F.C.I. 8929.....	23.3	25.2	22.4	6.1	20.0	10.0	10.0	10.0	5	0

^a The kernel smut used in 1929 consisted of physiologic forms 1 to 5, inclusive (MELCHERS, L. E., FICKE, C. H., and JOHNSTON, C. O. Op. cit.). Separate varietal plots were employed for each different smut used in 1929-32, inclusive. In 1930 the kernel smut consisted of a composite of forms 1 to 5, and the separate forms 1 to 5, inclusive. In 1931 only physiologic form 2 was used. In 1932 a composite of physiologic forms 1 to 5 and the separate forms 1, 2, and 3 were used.

^b The letters C. I., F. C. I., S. P. I., and K. B. indicate accession numbers, respectively, of the Division of Cereal Crops and Diseases, Division of Forage Crops and Diseases, and Division of Foreign Plant Introduction, all of the Bureau of Plant Industry, U. S. Department of Agriculture, and of the Department of Botany of the Kansas Agricultural Experiment Station.

^c The percentages derived from cutting back were determined by dividing the number of plants that produced smut after this treatment by the number of mutilated plants per row.

^d Killed by frost.

TABLE 1.—Percentage of kernel smut in mutilated sorghum plants as compared with the percentage on primary heads of nonmutilated plants; Manhattan, Kans., 1929-32—Continued

Group, variety, or selection	Accession no	Percentage of kernel smut on—									
		Primary heads, plants not cut back				Auxiliary heads of cut-back plants				Heads of secondary tillers of cut-back plants	
		1929	1930	1931	1932	1929	1930	1931	1932	1931	1932
Kafir × feterita—Continued											
Feterita × kafir	F. C. I. 6220			7.5	0			0	0	0	0
	F. C. I. 8951			5.5	8.3			0	10	5	(d)
	F. C. I. 8917	0.5	3.0		1.8	0	0	0	0	0	0
Kafir × milo hybrids											
26-3-1-1	K. B. 2561	7.9	14.6	16.5	4.1	0	0	0	0	10	0
	K. B. 2693	3.5	20.6	16.2	5.6	0	6.7	0	0	0	0
Dwarf Yellow milo × Pink kafir											
	K. B. 2694	4.3	9.5	6.7	5	5.0	13.3	5	5	0	0
	K. B. 2692	18.3	10.0			8.0	20.0				
	K. B. 3045			5.8	1.9			0	0	0	0
Kafir × milo 38-1-2-1	K. B. 2579	6.2	30.6			5.0	0				
Kafir × milo × Dwarf Yellow milo 31-79	K. B. 3032			17.3	2.4			0	0	0	0
(Kafir × milo) × Dwarf Yellow milo No. 6	K. B. 3033			0	1.6			0	0	0	0
Milo × kafir 8-2-6	K. B. 3031			1.7	13.0			(c)	0	0	0
Milo × feterita											
Hybrid	F. C. I. 8926	0	0			0	0				
Milo and milo hybrids											
Fargo Straightneck	C. I. 809	25.3	16.1	0	0	5.0	6.7	0	0	0	(d)
Dwarf Straightneck	K. B. 2844	5.3	2.2			0	0				
Open pollinated erect	K. B. 2845	6.5				0					
Dwarf Yellow	K. B. 2515	0	2.9	0	0	5.0	0	10	0	5	(d)
	C. I. 332	0	7.3	5.4	0	5.0	6.7	5	0	10	5
Standard White	C. I. 352	1.6	0			0	0				
Standard Yellow	C. I. 234	8.2	0			5.0	0				
Dwarf White	F. C. I. 8927	1.5	2.4			0	0				
Cream	K. B. 2569	2.5	4.5			0	0				
Double Dwarf	K. B. 3025			5.4	1.1			0	0	0	(d)
Sooner	K. B. 3027			21.7	0.9			0	0	10	0
Beaver	K. B. 3029			27.7	5.5			5	0	5	0
Early White	K. B. 3026			21.2	4.2			0	0	0	0
Early White No. 480 × Dwarf Yellow milo no 332	K. B. 3028			26.9	1.5			5	0	0	0
Wheatland	K. B. 3031			13.8	1.7			15	0	10	0
Kansas Orange sorgo × Dwarf Yellow milo											
Selection	K. B. 2680	39.6	6.5			5.0	6.7				
	K. B. 2681	0	0			0	0				
	K. B. 2684	30.1				0					
Kansas Orange × milo	K. B. 3039			19.1	3.2			5	0	0	5
Hegari											
Selection	K. B. 2518	0.2	0	0	0	0	0	0	0	0	0
	K. B. 2537	13.6	1.3	0	0	5.0	0	0	0	0	0
Broomcorn											
Standard	C. I. 583	37.8	18.1			18.0	20.0				
Acme	C. I. 243	38.9	44.2	13.6	8.2	10.0	15.0	0	0	0	(d)
Kaoliang											
Dwarf Shantung	C. I. 293	23.1	5.0			0	0	0	0	0	0
Manchu Brown	C. I. 171	28.4	22.0	7.8	5.3	0	0	0	0	0	0
Miscellaneous											
Wonder	K. B. 3010			5.1	6.7			15	0	10	0
	K. B. 3003			8.1	3.4			5	0	5	0
Darso	K. B. 2536	21.4	9.5			10.0	20.0				
White darso	K. B. 3002			3.4	9.4			0	0	0	0
Schrock	C. I. 616	9.2	5.7			10.0	0				
White durra	C. I. 81	31.1	19.8			5.0	6.7				
White Yolo	K. B. 2525	35.3	60.2	27.5	6.4	5.0	13.3	0	0	0	(d)
	C. I. 85	35.7	50.5	7.2	10.7	8.0	51.7	30	5	5	(d)
Shallu	K. B. 2579	38.6				13.0					
	K. B. 2981	6.4	7.6			0	0				
Sudan grass	K. B. 3040			3.5	3.8			10	0	0	(d)
Manko maize	K. B. 3044			32.5	6.8			10	0	0	0
Oklahoma Johnny	K. B. 3004			17.5	7.0			0	5	0	(d)
Sagrain	F. C. I. 8917			24.2	16.9			0	0	15	0
Chiltex											

* Killed by frost.

* 1 sucker.

It will be noted that such varieties as Leoti Red sorgo, Red Kafir, Dwarf Yellow milo, and shallu carry a high percentage of latent smut infection. Not all susceptible varieties, however, seem to carry large amounts and many carry very little. In 1929 out of a total of 176 heads of Leoti Red sorgo, 55 (31.3 percent) had primary head infection, and of the 20 normal heads cut back, 11 axillary heads (55 percent) developed smutted panicles. Had all of the 121 normal heads been cut back and the rate of smut infection on cut-back heads remained the same, there would have been 66.6 axillary heads and 55 primary heads, a total of 69.1 percent showing smutted panicles. On this basis it may be assumed that of the plants of Leoti Red sorgo actually invaded by the kernel-smut organism, only about half developed smutted panicles. The reaction of a resistant variety is not changed by cutting back, i.e., a resistant variety cannot be made susceptible. Resistance, therefore, is an inherited character, probably physiologic in nature.

The explanation for this reaction in sorghums is speculative, since the writer has not conducted histologic studies to demonstrate the presence of the smut fungus in the tissues of the host. From the studies that have been made it would appear that the following explanation may be the correct one.

The period during which infection by the kernel-smut fungus can occur is limited to 5 or 6 days immediately following the planting of the seeds. At this time, the infection hyphae evidently may gain entrance either to the meristematic tissue at the very apex of the growing point or to some of the embryonic cells a short distance back from the tip, where leaf and lateral bud primordia are beginning to differentiate. In the former case, short strands of hyphae would probably remain associated with the stem initials and the meristematic cells immediately behind them, and would also progress, through the agency of host cell division, to the cells in the basal portion of the meristematic region. As a consequence, not only the terminal inflorescence would be invaded and subsequently smutted, but there would also be a strong probability that the lateral buds, differentiating such a short distance behind would be similarly invaded. This might be tested by severing the inflorescences of diseased plants as soon as they emerge sufficiently from the boot to show that the smut mass is developing normally. If the supposition advanced above is correct, the disease should develop in the new panicles from lateral buds in every infected plant thus beheaded.

On the other hand, if the infection hyphae were to invade the meristematic region farther back where leaf and lateral stem differentiation are just taking place, and not in the apical cells, the division of the latter might result in the development of the growing point in advance of the infection hyphae and thus in its being able to grow away from the point of danger and produce a smut-free inflorescence. This would be particularly true if invasion of the meristematic tissues could be accomplished only by the division of cells containing mycelial fragments; otherwise, the hyphae would probably progress by intercellular invasion or direct penetration through cell walls, from the lateral cells of the apical meristem to the terminal cells. As Kolk⁸

⁸ KOLK, L. A. RELATION OF HOST AND PATHOGEN IN THE OAT SMUT, *USTILAGO AVERNÆ*. *Bul. Torrey Bot. Club* 57: 443-507, illus. 1930.

has demonstrated, these two types of invasion occur in oat smut, and as a result the growing point is soon thoroughly infested with hyphae even though the point of infection may have been some distance removed. This is particularly significant in view of the fact that she had no difficulty in obtaining 100 percent smut development from inoculated seed. Likewise, in bunt, smutted plants may be obtained readily by seed inoculation, and it has been proved ⁹ that the mycelium may penetrate between the cells and from cell to cell.

The total number of varieties of sorghum infected with each form of kernel smut, after being cut back, and the percentage of kernel smut produced by cutting back are shown in table 2. The outstanding feature to be noted is that form 2, the milo kernel smut, produced diseased heads in the largest number of varieties and also usually produced the highest percentage of infection of plants within a variety.

TABLE 2.—*The number of varieties of sorghum and the percentage range of covered kernel smut infection produced by the various physiologic forms of Sphacelotheca sorghi in beheaded sorghum*

Year and physiologic form no. ^a	Number of varieties in the percentage class indicated									Total number varieties infected with each form
	0-4 9	5-9 9	10-14 9	15-19 9	20-24 9	25-29 9	55-59 9	80-84 9	90-94 9	
1929										
1		2	2		1					5
2		9	5	3	1		1			19
3		7	2							9
4		1	1		1					3
5		2	2	1		1				6
1930										
1		1							1	2
2		4	2		1			1		8
3			1			1				2
4		3								3
5		1	1							2

^a Physiologic forms 1 to 5, inclusive, are the so-called kafir, milo, and 3 feterita forms of *S. sorghi*.

That form 2 should be more potent in this regard was not unexpected, for, as previous studies ¹⁰ had demonstrated, this form attacks not only all the varieties ordinarily susceptible to other forms but also the milos, some strains of hegari, and White Yolo, which are highly resistant to all other forms of kernel smuts to which they have been exposed in experiments at this station.

SUMMARY

Many sorghum plants among the susceptible varieties do not develop kernel smut in the primary heads even though the plants have the mycelium of *Sphacelotheca sorghi* within their tissues.

Cutting back infected plants that do not show kernel smut in the primary heads is often followed by the development of smut in the new growth of axillary shoots and in the shoots developed from sucker buds.

⁹ WOOLMAN, H. M. INFECTION PHENOMENA AND HOST REACTIONS CAUSED BY TILLETIA TRITICI IN SUSCEPTIBLE AND NONSUSCEPTIBLE VARIETIES OF WHEAT. *Phytopathology* 20: 637-652, illus. 1930

¹⁰ MELCHERS, L. E., FICKE, C. H., and JOHNSTON, C. O. *Op. cit.*

Highly resistant or immune varieties of sorghum do not develop kernel smut in heads produced after the plants have been mutilated as described, i.e., resistant varieties evidently do not carry smut hyphae in their tissues. A variety that is really susceptible may appear resistant if judged only by the heads of original or main tillers.

The increase in kernel smut obtained by plant mutilation is directly proportional to the degree of susceptibility of the variety as expressed by primary head infection. Thus if a variety is very susceptible to kernel smut as shown by primary head infection, the percentage of kernel smut obtained by cutting back apparently smut-free plants will be proportionately large.

Five physiologic forms of *Sphacelotheca sorghi* were used as inoculum for sorghum seed in these studies. Form 2, the milo form, not only attacked a greater number of varieties, including the usually resistant milo, hegari, and White Yolo, but also caused a higher percentage of kernel smut within a variety than the four other forms.

The phenomena of infection and invasion of sorghum plants by the kernel-smut fungus have not been studied histologically, but possible explanation of these phenomena, based upon the results obtained in this experiment, is given.

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EFFECT OF ENVIRONMENTAL AND CULTURAL FACTORS ON THE DWARF DISEASE OF ALFALFA¹

By J. L. WEIMER

Senior pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In an earlier article (14)² the writer has described briefly, under the name "dwarf", a previously unreported disease of alfalfa (*Medicago sativa* L.), the cause of which is unknown. The fact that the dwarf disease is limited to the southern half of the State of California suggests the possibility that there may be some climatic conditions which tend to limit its distribution or some cultural factors which affect its severity. Temperature, soil moisture, soil fertility, and cutting in an immature stage are factors that appear to be worthy of consideration. It has been suggested by some that the disease might be caused by one or more of these factors.

It is the object of this paper to report experiments and observations designed to show what effect, if any, certain cultural and environmental factors may have on the development of the dwarf disease and on the longevity of alfalfa stands in southern California.

METHODS AND MATERIAL

For these investigations, 16 plots, each 1 by 4 rods in area, were laid out in a 1-year-old alfalfa field on the experimental farm of the University of California at Riverside, Calif. Although this field had been dry-farmed to cereal crops for a number of years, it had never grown alfalfa. The alfalfa used was the so-called Chilean variety, which is most commonly grown in southern California. When the plots were laid out, on July 1, 1929, the stand was in excellent condition in regard to both number and growth of plants. Since the ground was only fairly level, the distribution of water had never been quite even; however, neither the stand nor the vigor of the plants was in any way impaired. This semidrought condition was easily remedied by a revised system of irrigation instituted for the plots, which were so arranged that each could be irrigated separately whenever desired. Figure 1 shows the arrangement of the plots in two series, of eight plots each, separated by an irrigation ditch and bordered on the east by another irrigation ditch. In each series border checks, each 4 feet wide, separated the plots into four groups of two each; the individual plots in each group were separated by an embankment. The two plots of each group were always irrigated alike. Since the "run" was only 4 rods long, it was possible to cover

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² Reference is made by number (italic) to Literature Cited, p. 367.

any plot quickly and evenly. Usually the water was allowed to remain on the plot for 4 hours after the ground had been entirely covered. By means of a soil auger it was found that this was sufficient time to soak the soil to a depth of 3 feet or more. Plots 1 to 4 and 9 to 12, inclusive, were irrigated once a month, and the remaining plots twice a month. However, plots 1, 2, 5, 6, 11, 12, 15, and 16 were allowed to go without irrigation during the seed-producing period each summer (about $3\frac{1}{2}$ months).

EFFECT OF SOIL MOISTURE

Early in these investigations field observations indicated that soil moisture exerted a very decided influence on the development of the

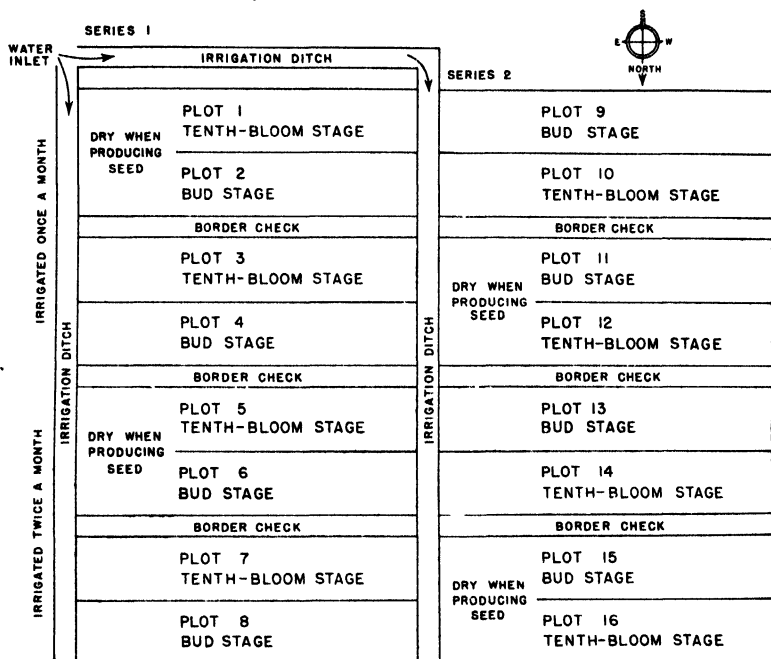


FIGURE 1. Diagram showing arrangement of plots used in experiments.

dwarf disease and consequently on the length of life of the alfalfa stands. It was noted that the alfalfa stands which died out first were those that received the most irrigation water or those in low spots where the water collected. Often a stand was very good in the high spots of a field, where water reached only with difficulty or sometimes not at all, but elsewhere it was quite thin. Likewise, in certain small valleys where growers do not irrigate their alfalfa but depend entirely on winter rainfall for their moisture supply, dwarf is practically nonexistent and the stands continue for many years.

Having been convinced from field observations that soil moisture is a factor in the development of dwarf, the writer sought confirmatory experimental evidence based on the stands in plots given various irrigation treatments.

The stand was determined by counting the plants in three 3-foot-square quadrats in each plot, one at the center and the others about

a third of the distance from each end. These quadrats were permanently located at the beginning of the experiment by driving a stake at each corner. Although the quadrats for the most part were fairly representative of the conditions, in a few cases, where the plants on one side or corner of the plots died more rapidly than those in the quadrat, the count taken was too high. This was especially true of plots 1 and 9, in which the plants died more rapidly along the south side than elsewhere; in fact by the end of the third season the stand in these plots was so poor that the yield figures were considered valueless.

TABLE 1.—*Effect of irrigation treatments on alfalfa stands from September 1929 to October 1931*

[Data represent averages from three 3-foot-square quadrats in each plot]

Number of irrigation treatments per month	Plot no.	Average number of plants					Percentage of plants (October 1931)—	
		September 1929	April 1930	September 1930	March 1931	October 1931	Dead	Living
	a b 1	45	40	35	24	14	69	31
	c b 2	76	68	56	44	35	54	46
	a 3	51	55	47	44	40	22	78
	c 4	54	53	40	38	13	76	24
	c 9	69	63	42	46	10	86	14
	a 10	62	58	47	48	41	34	66
	c b 11	68	58	41	46	40	41	59
	a b 12	57	52	49	40	39	32	68
	a b 5	67	69	52	52	53	21	79
	c b 6	68	62	47	48	45	34	66
	a 7	73	57	50	48	12	84	16
	c 8	60	45	37	40	0	100	0
	c 13	84	58	54	52	6	93	7
	a 14	80	54	48	44	14	83	17
	c b 15	89	59	65	58	55	38	62
	a b 16	76	55	55	50	43	43	57

a Plants cut in tenth-bloom stage

b Plants dry when producing seed.

c Plants cut in bud stage

The number of plants in the different plots, as represented by the average of the three quadrats, is given in table 1. The difficulty involved in making accurate counts of alfalfa plants is appreciated by those who have attempted it. Sometimes two or more plants are growing so close together that it is impossible to tell how many are present without removing considerable soil from about the crowns. In the present instance the soil was not removed, but every quadrat was counted by two men, each counting the entire quadrat, and the average of the two counts was used. In case the counts differed by more than a few plants, a recount was made by one or both men. Counts were made in the spring and in the autumn of each year. The results of these counts are presented in table 1. The number of plants per square foot in September 1929 ranged from 5 in plot 1 to 9% in plot 15. For the most part the figures show that there was a gradual thinning of the stand, varying in rapidity in different plots, so that by October 1931 some plots were still quite good while others had few or no plants left. At that time the stands in plots 1, 3, 4, 7, 8, 9, 13, and 14 were very thin, appearing even thinner than the percentage of living plants recorded in the table would indicate.

As is often the case, the stands did not always thin out uniformly; frequently there were more than the average number of plants in a quadrat, thus making the average higher than it would have been had it been possible to count all the plants in the plot. For example, table 1 shows 78 percent of a stand left in plot 3 in October 1931, while estimates made at the same time indicated that there was about 20 percent of a stand at one end and 70 percent at the other end of the plot, or an average of about 45 percent for the plot as a whole. On the other hand, certain quadrats thinned out more than the average of the areas they represented.

In view of the fact that a gradual decrease in the number of plants in each plot took place during the 3 years the experiment was in progress, it becomes of interest to consider the reasons for the unequal rate of thinning. As already stated, some plots (1 to 4 and 9 to 12, inclusive) were irrigated once a month during the growing season, whereas the others were irrigated twice a month, and plots 1 and 9 thinned out more rapidly along the south side than did the others; but since other factors, especially a difference in soil type, were involved in plots 1 and 9, these two plots are not strictly comparable with the rest and are therefore omitted from the present discussion. The remaining figures show an average loss of 43 percent for the plots irrigated once a month and 62 percent for those irrigated twice a month. Another factor is involved, however, the fact that plots 2, 5, 6, 11, 12, 15, and 16 were not irrigated during the seed-growing period. For the plots similarly irrigated the following averages were obtained: The average percentage of loss in stand for the plots irrigated once and for those irrigated twice a month throughout the season was 44 and 90, respectively, as compared with 42½ and 34, respectively, for the plots similarly treated but left unirrigated during the seed-producing period. These figures show that there was a decidedly more rapid thinning of the stand in the plots irrigated twice a month, which, however, was counteracted when the stands were kept dry during the seed-producing season (figs. 2, 3, and 4). The difference in the rate of thinning of the two groups of plots that were allowed to go to seed is probably not significant.

Figures 2, 3, and 4 show the condition of certain of the plots in October 1931, when the experiment was discontinued. There seems to be a discrepancy between the data for plot 3 given in table 1 (78 percent of plants in quadrats living in October 1931) and the appearance of the plot in figure 2. This is probably due, at least in part, to the fact that the number of plants in the quadrats was not truly representative of the condition of the plot as a whole. Plot 3 was one of the best plots throughout the experiment and yielded well (table 3). However, during the late summer and autumn of 1931 the plants died out quite rapidly.

The conclusion seems justified that under the conditions of this experiment the rate of thinning of the alfalfa stand was greatly increased, in fact was practically doubled, by doubling the number of irrigations, provided the stands were not allowed to go without irrigation during the summer months. This experiment, therefore, seems to confirm the field observations indicating that soil moisture is an important factor in the longevity of the stands of alfalfa in southern California.

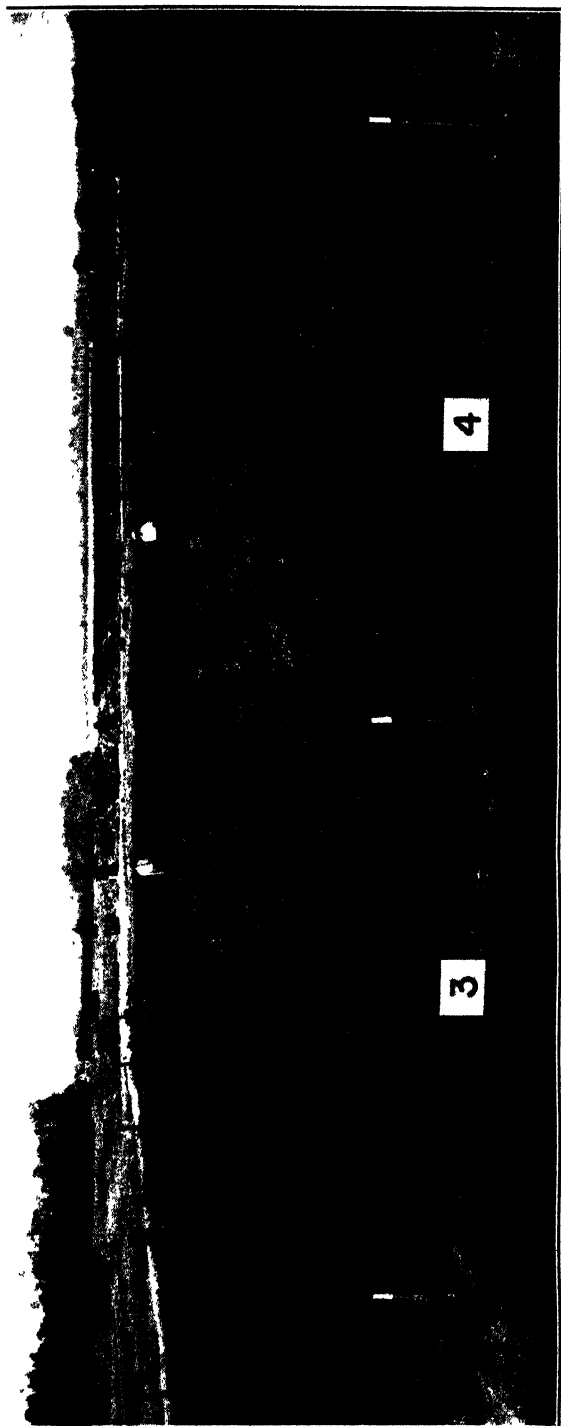


FIGURE 2.—Several alfalfa plots, showing the effect of different cultural treatments. In foreground, plots 3 and 4, irrigated once a month and cut regularly for hay; vegetation mostly weeds. At left, part of plots 1 and 2; at right, part of plots 5 and 6. In background, several plots of second series (9 to 16, inclusive); the two men shown are standing on bank of irrigation ditch separating the two series of plots. Just back of plots 3 and 4 are plots 11 and 12. Plots 1, 2, 5, 6, 11, 12, 15, and 16 were left unirrigated each summer during growth of seed crop. To right of plots 11 and 12, plots 13 and 14, still farther to right, plots 15 and 16 (For details of arrangement and for treatment given each plot, see figure 1.) Photographed in October 1931

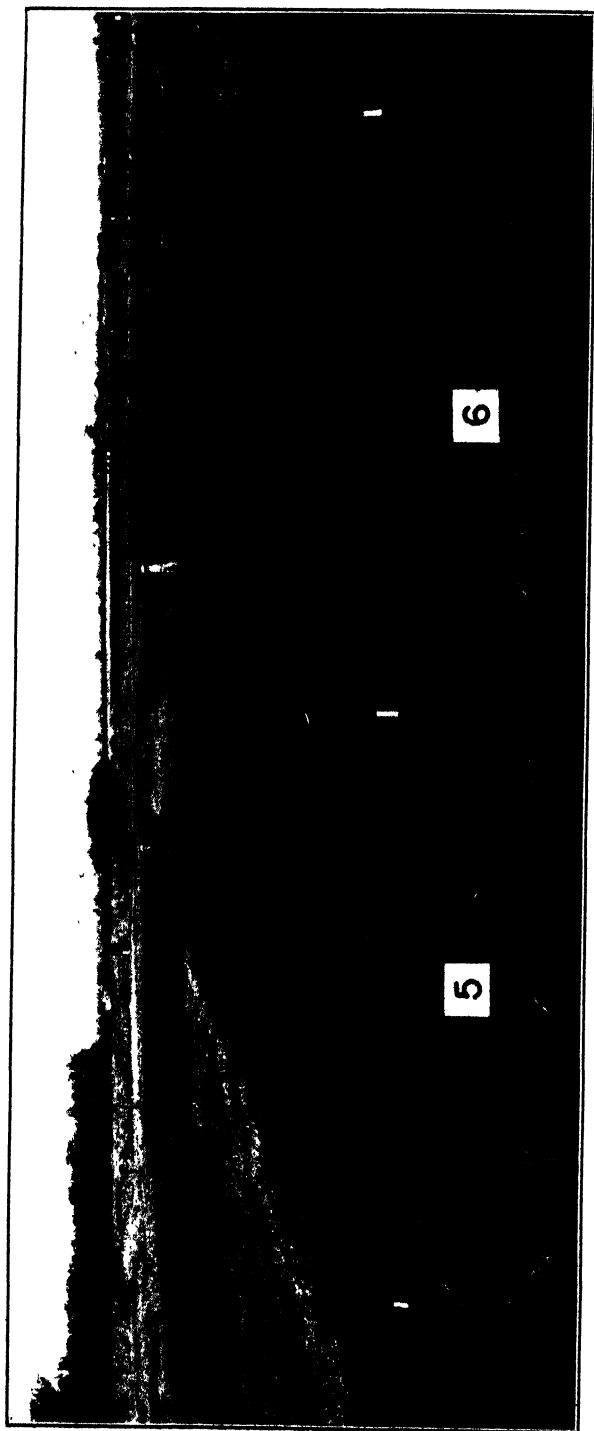


FIGURE 3.—Closer view of plots 5 and 6, showing condition of stand in plots irrigated twice a month except when growing seed. Photographed in October 1931.

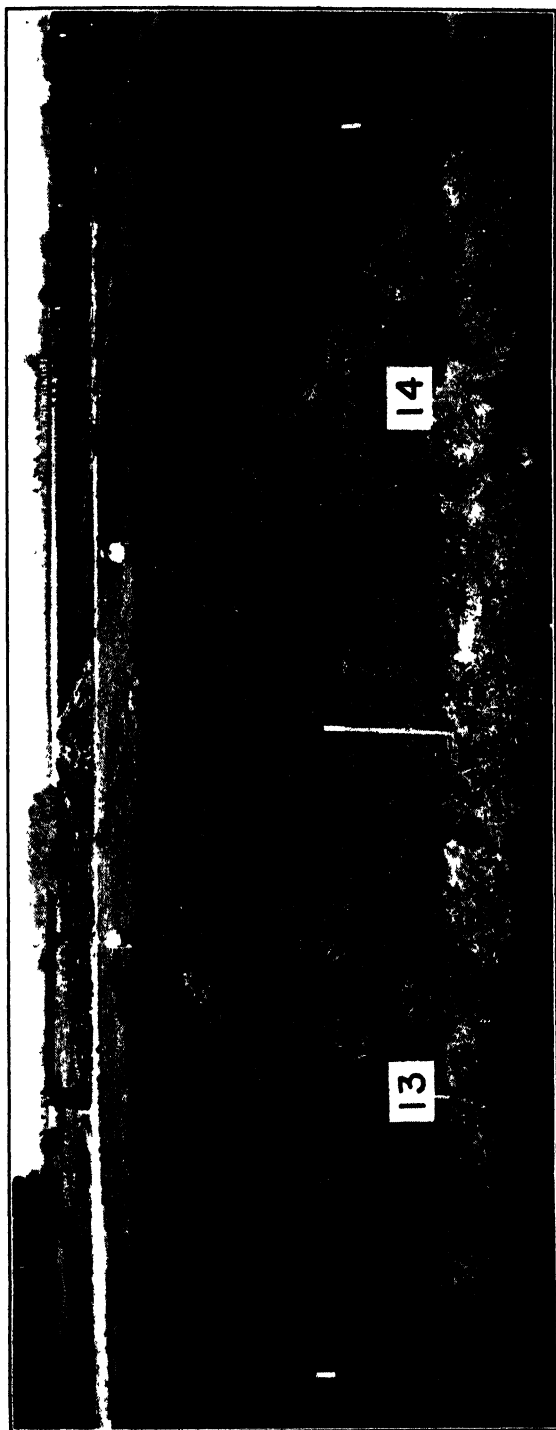


FIGURE 4.—Closer view of plots 13 and 14, showing condition of stand in plots irrigated twice a month and cut regularly for hay. Vegetation mostly weeds, only a few scattered alfalfa plants. Compare with stand of alfalfa in plots 11 and 12 (left) and 15 and 16 (right), where seed was produced each summer. Photographed in October 1931.

EFFECT OF FREQUENCY OF CUTTING

In southern California it is a common practice to cut alfalfa before the blossoms open, in order to meet the market demand for such hay to supply cattle and rabbit feed. This practice is believed by some to be responsible for the rapid thinning of the stand. Several investigators have shown (3, 8) that cutting alfalfa when too immature shortens the life of the stand somewhat, at least under certain conditions. An experiment was therefore designed to ascertain whether cutting in the bud stage, as compared with cutting in the tenth-bloom stage, makes any appreciable difference in the longevity of the stand. This part of the experiment was undertaken in conjunction with the study of soil moisture recorded herein, and the discussion must be based on the figures given in table 1. In this experiment, as shown in figure 1, every other plot was cut in the tenth-bloom stage and the alternate ones in the bud stage, except when the stands were allowed to go to seed. In the first series (fig. 1) the odd-numbered plots were cut in the tenth-bloom stage and the even-numbered plots were cut in the bud stage; in the second series the order was reversed.

As already stated, plots 1 and 9 are not strictly comparable with the rest; hence they and their companion plots (2 and 10) are omitted from this discussion. Since the plots run in groups of two, perhaps the best way to consider them is in that order. For example (table 2), plot 3, cut in the tenth-bloom stage, and plot 4, cut in the bud stage, have lost 22 and 76 percent, respectively, of their original stands. In like manner, plots 5, 7, 12, 14, and 16 may be compared with their companion plots, 6, 8, 11, 13, and 15, respectively. Such a comparison indicates that for the most part there is a slightly greater loss of plants in plots cut in the bud stage than in the other plots. The reverse is true for plots 16 and 15; however, the small difference is probably not significant. It is also noticeable that (omitting plots 16 and 15) the differences, though always in the same direction, are not large, except that between plots 3 and 4. The wide difference between these two plots is probably due to the fact that the figure for plot 3 is not representative of the plot as a whole, as previously explained. A further study of table 1 shows that the plots which thinned out so completely did so largely during the summer of 1931. Further light on just when this rapid dying of the plants occurred is given by the yield data in table 3, which show that all the plots, except plot 1, yielded fairly well for the first three cuttings. A falling off in yield is noticeable in plots 4, 7, and 9, in the fourth cutting, and a decided reduction is evident in plots 4, 7, 8, 9, 10, 13, and 14, in the fifth cutting, which was made between June 19 and July 29. It seems evident from these data, as well as from many field observations, that the rapid reduction in stand starts about the middle of June and, if conditions remain favorable for the development of the disease, continues until the stand is nearly or entirely depleted. Doubtless the most rapid dying of the plants takes place during July and August.

Just why the yields of plots 3 and 10 should have increased so greatly at the fourth cutting is not evident from the data at hand. These plots, as well as several of the others, showed more or less decrease in yield at the third cutting, followed by an increase at the

fourth cutting. It seems to the writer most probable that soil moisture was at least one of the major factors involved in these fluctuations. This is suggested by the fact that the greater variations occurred in the plots irrigated only once each month. Occasionally the water was not available when needed, so that the plots irrigated only once a month suffered somewhat from drought.

TABLE 2.—Effect of cutting plants at different stages of maturity on percentage of loss in stand

Stage when cut	Plot no.	Percent- age of plants dead	Stage when cut	Plot no.	Percent- age of plants dead
Tenth bloom	3	22		4	76
	5	21		6	34
	7	84		8	100
	12	32		11	41
	14	83		13	93
	16	13		15	38

The total yields for the year are not of much value, since they are influenced by two inseparable factors, namely, thinning of the stands and weakening of the plants, the latter due, no doubt, to the reduction of root reserves as described by Graber and his coworkers (3). This weakening, which was evident as a shortening of top growth, was quite noticeable at times, especially during the latter part of the season, even before the stand started to thin so rapidly. However,

TABLE 3.—Yield (in pounds) of weed-free hay for each cutting and the total yield for 1931

Plot no.	Pounds of weed-free hay from indicated cutting							Total
	1	2	3	4	5	6	7	
1.....	3 3	(a)	(a)	(b)	(b)	c 52 57	27 47	83 34
2.....	23 8	50 46	58 17	(b)	(b)	c 78 47	60 66	277.56
3.....	32 1	86 00	52 26	96 96	85 00	52 52	(d)	404 84
4.....	21 8	38 25	43 53	34 84	16 36	0	3 73	158.51
5.....	39 7	104 19	93 93	(b)	(b)	c 75 15	62 71	375 68
6.....	36.1	75 48	60 05	(b)	(b)	c 70.19	48 20	280 02
7.....	31.5	93 12	71 08	21 81	1 90	0	0	219 50
8.....	20 6	34 77	34 10	40 29	3 00	0	0	132 76
9.....	14 2	51 07	51.26	39 23	17 59	0	0	173.35
10.....	27 1	91 64	58 01	86 10	33 84	55 35	(d)	362.04
11.....	18 3	64 94	61 94	(b)	(b)	c 86 88	63 67	295.73
12.....	31 7	92 37	66.35	(b)	(b)	c 97.53	67 34	355 29
13.....	23 7	36 54	49 98	54 28	2.50	0	0	167 00
14.....	28 3	83.70	66 63	75 41	38 08	0	0	292 12
15.....	33 3	69 37	66.75	(b)	(b)	c 112 67	62 18	344 27
16.....	44.1	115 46	90 04	(b)	(b)	c 120.74	63 48	433.82

a Not weighed, owing to large quantity of weeds

b Plots allowed to go to seed.

c Weight of straw from seed crop.

d There was no seventh tenth-bloom cutting.

table 4, which gives the total yields by plots and, like table 2, is so arranged that the adjacent plots of each group can be compared readily, shows that in every case the plots cut in the tenth-bloom stage yielded considerably more hay than the adjacent plots cut in the bud stage. The total yield for all the plots cut in the tenth-bloom stage is approximately one third greater than that for the plots cut in the bud stage.

TABLE 4.—Total yields (pounds) of weed-free hay in 1931 from plants cut in the tenth-bloom stage and from those cut in the bud stage

Stage when cut	Plot no	Yield	Stage when cut	Plot no.	Yield
Tenth bloom..	3	404.84	Bud	4	158.51
	5	375.68		6	290.02
	7	219.50		8	132.76
	12	355.29		11	295.73
	14	292.12		13	167.00
	16	433.82		15	344.27
Total		2,081.25	Total		1,388.29

DWARF AS A FACTOR IN RAPID THINNING OF STANDS

It has been pointed out how soil moisture and possibly to a certain extent frequency of cutting may tend to shorten the life of the alfalfa stands. The chief object of the present experiment, however, was to determine, if possible, what effect these factors may have on the rate of spread and on the severity of dwarf. The percentage of diseased plants in each plot was determined in the spring and autumn of each year of the experiment; the figures thus obtained are given in table 5. For the first three counts 25 plants, irrespective of whether they were diseased or healthy, were dug from each corner of the plot. It was then decided that a more representative sample could be obtained by digging all the plants in two strips, each 1 foot wide, extending across the plots and located about one third of the distance from either end of the plots. Since the strips in which the counts were made in November 1931 were adjacent to those used in February 1931 they were comparable in every way. The number of plants obtained from each plot by this method ranged from 50 to 125, depending upon the thickness of the stand. Every root was peeled and examined, so that the early stages as well as the late stages of the disease were observed.

TABLE 5.—Percentage of diseased alfalfa plants in each of the 16 plots on various dates

Plot no.	Percentage of diseased alfalfa plants on indicated date				
	October 1929	April 1930	September 1930	February 1931	November 1931
1.	53.1	36.2	36.0	44.6	(a)
2.	21.9	10.3	33.0	16.4	17.7
3.	13.3	10.5	20.0	12.4	63.0
4.	4.0	10.9	30.0	22.1	26.0
5.	0	5.1	4.0	3.0	37.0
6.	4.4	4.8	24.0	11.7	36.0
7.	8.5	16.2	64.0	65.3	(a)
8.	0	16.0	50.0	77.5	(a)
9.	6.2	17.0	42.0	52.3	(a)
10.	0	6.9	15.0	16.3	31.5
11.	2.5	1.0	6.0	13.4	25.5
12.	1.2	1.0	7.0	6.8	30.0
13.	5.3	3.0	31.0	50.1	41.7
14.	5.3	6.0	19.0	36.6	40.0
15.	2.6	2.9	3.0	4.1	10.4
16.	0	1.0	7.0	12.7	25.0

* Stand so depleted that there were not enough plants left to make any reliable counts.

The data in table 5, column 2, show the amount of disease present in October 1929, when the stand was a little less than 2 years old. It will be observed that there was considerable disease in plots 1 to 3. The presence of the disease can be explained by the fact that these plots were nearest to the source of water and had received considerably more water than the others at each irrigation before the experiment was started. It is interesting to note that as high as 53 percent of diseased plants were present in plot 1 in the second year of the stand on soil which had never before grown alfalfa and which was several miles from any other field of growing alfalfa. It should be stated, however, that another alfalfa field, about half a mile away, had been plowed in the autumn after the plots under discussion were seeded. Nevertheless, it is difficult to see how this could have been of much importance as a factor, for the irrigation water did not come in contact with the old stand on its way to the new one and the prevailing winds blew in another direction.

Except in plots 1 to 3, there was comparatively little disease in October 1929. The following spring more diseased plants were found; yet, with the exception of plot 9, they were confined largely to the east end of the series on the east, including plots 1 to 8. From that time the disease spread so rapidly that by November 1931 some plots had only an occasional plant left.

From the data in table 5 it may be determined whether a correlation existed between either soil moisture or frequency of cutting and the development of the disease. It must be borne in mind that plots 1 to 4 and 9 to 12, inclusive, were watered once a month and the others twice, and that plots 1, 2, 5, 6, 11, 12, 15, and 16 were without irrigation during the seed-growing period each summer. As noted previously, plot 1 had a high percentage of diseased plants at the beginning (table 5); and, as indicated by the yield data in table 3, the stand died out rapidly. Although the northwest corner remained fairly good throughout, for the most part plot 1 did not show any noticeable response to the treatments given it; it seems best, therefore, to omit plot 1 from the comparisons that follow. It should also be pointed out that some of the figures for November 1931 may be misleading. For example, plot 4 seems to have had less disease in November than plot 3, although the reverse had been true previously. This difference is due to the fact that not enough plants were left in plot 4 in November 1931 to make a representative sample; hence this figure had little or no significance. A comparison of each group of companion plots (3 and 4, 5 and 6, etc.) shows few very striking differences. The outstanding difference apparent between plots 9 and 10 can be explained by the fact that in plot 9, as in plot 1, the plants died more rapidly from the start than in the plot adjacent to it. In many cases the plots cut in the bud stage had a higher percentage of dwarf than the corresponding plots cut in the tenth-bloom stage. There were several instances, however, where the disease was equally prevalent in both plots or where the plot cut in the tenth-bloom stage was the more seriously affected. In February 1931 plots 4, 6, 8, 11, and 13, which were cut in the bud stage, had higher percentages of disease than did the adjacent plots (3, 5, 7, 12, and 14). The reverse is true of plots 15 and 16. In September 1930 plot 7 had more disease than plot 8, and plot 16 had more than plot 15. These

figures fail to give convincing proof that the disease is very greatly influenced by the stage of maturity of the plants when cut; hence it is difficult to see how cutting in the bud stage could be responsible for the dwarf disease.

The correlation between soil moisture and the prevalence of dwarf may be determined from the counts made in February 1931. These counts seem to be as suitable as any, if not the most reliable of all, since the samples were taken in two uniform strips across the plots at a time when the disease was very prevalent but when the stands, except that of plot 1, were not too thin. For the plots (except 1 and 9) of the series irrigated once a month, the average percentage of disease was 14.6; and for those irrigated twice a month, 32.6, a difference of 18 percent. This difference is even greater when the plots allowed to go to seed are excluded, the averages being 16.9 and 57.4 percent for the plots irrigated once and twice a month, respectively, or a difference of 40.5 percent. If those plots which had exactly the same treatment are compared, the respective percentages of diseased plants will be found to be as follows: Plots 2 and 11, 16.4 and 13.4 percent; plots 3 and 10, 12.4 and 16.3 percent; plots 5 and 16, 3.0 and 12.7 percent; plots 6 and 15, 11.7 and 4.1 percent; plots 7 and 14, 65.3 and 36.6 percent; and plots 8 and 13, 77.5 and 50.1 percent. These figures show a fairly consistent amount of disease in the duplicate plots. They also emphasize the great increase caused by the added amount of water when the applications were made throughout the growing season, and show the effect on the wet series of the summer drought, which seemingly had no effect on the dry series.

Although only one experiment is involved, the results with respect to the effect of soil moisture on dwarf are so striking and consistent and check so closely with field observations that the following conclusions seem to be justified: (1) That the alfalfa dwarf disease is greatly influenced by soil moisture; (2) that high soil moisture during the summer months is conducive to the spread of dwarf; and (3) that the reverse is true of low soil moisture. The exact degree of soil moisture conducive to the increase or decrease of dwarf has not been determined. Seed production, with the attendant dry period, has a tendency to retard the spread of the dwarf disease, especially if the soil moisture has been high at other times.

The question now arises as to whether soil moisture caused the decrease in stand through its effect on the development of dwarf; that is, whether dwarf was the sole or at least the major cause of the premature thinning of the stands in these alfalfa plots. Probably this question can best be decided by comparing tables 1 and 5. The percentage of plants lost in plot 3, as given in table 1, was rather low; the percentage of disease in the same plot, as given in table 5, was comparatively low until November 1931. Nearly all the plants indicated as diseased in November 1931, however, had an early stage of dwarf; hence they would not have died until the following year. The correlation does not seem to hold for plot 4, in which the percentage of plants lost was high (table 1) and the percentage of disease was comparatively low (table 5). In most of the other plots there seems to be a fairly close correlation between the plants lost and the amount of dwarf. Owing to errors in sampling, which may be fairly high in some cases, a considerable variation must be expected. Furthermore, it seems probable that some of the plants that died

were not affected by dwarf. In fact, it is known that some plants were so severely affected with a crown rot that no tops were formed, although the roots still appeared to be healthy. On the other hand, the roots of a considerable number of plants made little growth. Although such plants were hardly ever affected with dwarf, many no doubt eventually died. In general, however, it is thought that the majority of the plants that were lost died from dwarf.

EFFECT OF TEMPERATURE

No definite experiments have been conducted to determine the influence of temperature on dwarf. Field observations, however, tend to refute the supposition that temperature is an important factor in the spread or the severity of the disease. Dwarf has been found to be as severe in places near the coast, where the temperatures are comparatively low, as in the interior valleys, where they are considerably higher. For example, temperatures recorded during June, July, August, and September, for 42 years in Los Angeles and for 23 years in Riverside (12), show that the mean and the mean maximum temperatures for Los Angeles were 5° and 10° F. lower, respectively, than those for Riverside. With respect to both the mean and the mean maximum temperatures more extreme conditions doubtless exist in many alfalfa fields, since alfalfa dwarf is found in places much nearer the ocean than Los Angeles, as well as in places where the mean temperatures are higher than those officially recorded for Riverside. That seasonal temperatures do affect the development of dwarf in the plant is indicated by the fact that the diseased wood in an affected plant is commonly overgrown during the winter months. As early as the 1st of December a thin white layer of new wood covering the yellow diseased area may be found in many plants. New wood continues to develop during the winter, so that by spring it may be a millimeter or more in thickness. When hot weather returns the disease again becomes active, involving the new wood, and the plant eventually dies.

Further knowledge of the effect of temperature on the development of dwarf must await a better understanding of the cause of the disease. So far as can be determined from a study of the records of the areas where dwarf is known to exist, temperature is probably not a limiting factor in the spread of the disease. Neither can temperature be considered a causal factor, since in localities where dwarf does not occur temperatures are found that are higher or lower than temperatures in localities where dwarf is known to be most destructive.

EFFECT OF SOIL FERTILITY

Many investigations have been conducted to determine what effect soil fertility has on the susceptibility of plants to disease. The review of the literature on one small group of diseases, by Stakman and Aamodt (11), shows the consensus of opinion to be that nitrogenous fertilizers increase the susceptibility of cereals to rusts whereas potassium and phosphorus salts tend to decrease it. These writers state that there is no very close agreement among investigators as to the exact way in which fertilizers effect a change in the resistance of a plant to rust. Stakman and Aamodt consider the effect to be largely indirect, through changes in the growth rate, density of stand, date of maturity,

and yield. Much has been written regarding the correlation between the rapidity of growth of fruit trees and their susceptibility to fire blight. Hoffer and Carr (6) have shown how the susceptibility of corn to root rot is influenced by the accumulations of iron and aluminum in the nodes, and how the availability of these compounds in the soil may be influenced by soil moisture and the presence of sufficient amounts of other salts such as calcium and phosphorus. It has also been shown in recent years (1, 2, 4, 7, 9, 13) that many soils are deficient in certain elements, such as copper, manganese, zinc, and boron, which are needed in very small amounts by plants but which may be quite toxic, producing conspicuous symptoms, if excessive amounts are present in the soil. The results of such investigations serve to emphasize the fact that soil fertility may affect disease in plants by increasing or decreasing the growth rate or by weakening the tissue, as in the case of iron and aluminum, and that a deficiency or an excess of certain elements may produce a condition in plants resembling that caused by micro-organisms.

In 1929 experiments were initiated to determine whether the addition of certain commonly used fertilizers would influence the severity of the dwarf disease or whether the affected plants would outgrow the disease when supplied with certain elements that might be lacking.

The first experiment was designed to show whether the affected plants would outgrow the disease when they were removed from the field and planted in cans containing soil to which manure or commercial fertilizers had been added. For this experiment subsoil was taken from a mountain side where no cultivated crop had ever grown. Six 16-gallon garbage cans were used; two of them, containing only the soil, served as controls; two others were filled with soil mixed with a nearly equal amount of fresh horse manure; and the remaining two received an application of a complete fertilizer (consisting of ammonium sulphate, nitrate of soda, blood, triple superphosphate, and sulphate of potash), which supplied 7.5 percent nitrogen, 9.25 percent P_2O_5 , and 4.75 percent K_2O , at the rate of 250 pounds per acre. The soil that was used supported vigorous growth without the application of any fertilizer. Eight 2-year-old alfalfa plants having an early stage of the disease were planted in each can. The experiment was set up in October 1929.

The plants used would normally have died during the summer of 1930. Observations made on October 22, 1930, showed that three of the control plants were alive but that their top growth was only about one third as tall as that of healthy plants growing in nearby cans and cut at the same time. A like number of plants in the cans to which manure had been added were alive and these too were very much dwarfed. Although the top growth was little if any better, the plants in the soil to which commercial fertilizer had been added did not die quite so rapidly, a total of seven plants being alive after 1 year. After 2 years one plant still remained alive in one of the cans treated with manure and one was alive in the soil to which commercial fertilizer had been applied. Just how long some of the control plants might have lived was not determined, as these plants were removed on December 2, 1930, and the cans used for another experiment.

It was concluded from this experiment that none of the treatments appreciably affected the rate of dying or the growth of the plants.

It is of course true that the experiment was carried out on a very limited scale; therefore one would probably not be justified in drawing any conclusions from it if the results had not been confirmed by field observations and other experimental data.

Although no fertilizer tests were conducted in the field, the writer had the privilege of observing and taking notes on such a test conducted during the years 1929-31, inclusive, by M. M. Winslow, farm adviser, of Riverside County, Riverside, Calif. In this experiment there were eight plots, each 50 by 80 feet in area, representing in duplicate three different treatments and a control. The rate per acre and the fertilizers applied were (1) 750 pounds of superphosphate, (2) 200 pounds of sulphur and 600 pounds of lime, and (3) 750 pounds of superphosphate and 500 pounds of potassium chloride. Since no difference in the vigor of the plants was observed, no yield data were taken.

TABLE 6.-- *Number of healthy and diseased alfalfa plants in plots receiving different fertilizer treatments*

Fertilizer treatment		Alfalfa plants in--						Average percentage of diseased plants
Plant food	Quantity per acre	Plot 1			Plot 2			
		Healthy	Diseased	Total	Healthy	Diseased	Total	
	<i>Pounds</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Percent</i>
Superphosphate	750	226	64	290	199	65	264	23.4
Sulphur	200	145	66	211	141	54	195	29.5
Lime	600							
Superphosphate	750	186	64	250	216	54	270	22.8
Potassium chloride	500							
Control		214	46	260	204	44	248	17.7

The conclusion reached by Mr. Winslow and the writer, namely, that none of the fertilizers had any appreciable effect on dwarf, is supported by the data presented in table 6, which were obtained in February 1931 by digging all the plants in two 6-inch strips extending across all the plots and located about one third of the distance from either end. Further counts were to have been made in the autumn, but the stand was so thin by that time that it was not deemed worth while. Table 6 gives the number of healthy and diseased plants and the average percentage of diseased plants in the duplicate plots. These values show a very small range in the percentages of diseased plants in the plots. There was practically no difference between the plots receiving superphosphate and those receiving both superphosphate and potassium chloride. The value for the lime-and-sulphur plots (29.5) probably is not significantly greater than the values for the other plots. On the other hand, the percentage of disease in both control plots was the lowest of all. In none of the plots was the ground quite level, so that at times there was a difference in soil moisture; this may account for some of the variation. Taking everything into consideration, it seems probable that none of the fertilizers had an appreciable effect on the disease; certainly they did not prolong the life of the stand. By the end of the third season the stand was so uniformly poor that it was no longer worth retaining.

As stated previously, it is known that certain chemicals, ordinarily used by plants in small amounts, may have a profound effect upon them. In order to determine whether such chemicals would affect the susceptibility of alfalfa plants to dwarf, three experiments were conducted in which the following chemicals were tested: Boron, manganese, strontium, bromine, titanium, iodine, aluminum, copper, and zinc. The use of these chemicals was suggested largely by an article by Haas and Reed (5) and also by Dr. Haas in a personal consultation. Chemically pure chemicals were used. For the most part these were sulphates or nitrates; boron, however, was applied as sodium borate, and iodine as potassium iodide. Magnesium sulphate and ammonium nitrate were also added to this mixture. Fifty grams of boron, 1 g of titanium, 1 g of iodine, and 454 g of other substances were applied to each of two plots, each 15 by 40 feet in area, in February 1930. In one plot, which was beginning its second year, about 10 percent of the plants were diseased; in the other, which was beginning its fourth year, about 40 percent of the plants were diseased. As a further control, a heavy application of cow manure was made on a portion of the field adjacent to the plot having the older stand. In each case there was plenty of alfalfa growing on adjacent untreated land to serve as a control. In order that the counts of the plants might be made from the same area each time, three permanent quadrats, each 3 feet square, were staked off in the plot having the younger stand. Very early in the season it became apparent that an unfortunate choice of location had been made for the plot having the older stand; hence only general observations were made upon this plot throughout the season. Careful observations throughout the summer showed no apparent difference between the areas to which the chemicals and the manure had been added and the control areas.

In the plot having the younger stand counts of the quadrats showed that an average of one third of the plants had died during the year. The loss of plants was irregular; 50 percent disappeared at one end of the plot and only 7.5 percent at the other. In February 1931 counts made of representative areas in the plot showed that an average of 40 percent of the remaining plants had dwarf. The plants died so rapidly during the summer of 1931 that comparatively few were left by autumn; hence no further counts were made. It was clearly evident that the chemicals had not increased the longevity of the stand. At no time was there sufficient difference between the plot and the surrounding stand to enable one to locate the former except by the stakes at each corner.

At the time that these two plots were laid out a small experiment was begun in which two 16-gallon garbage cans were filled with soil from one badly diseased field and two with soil from another badly diseased field. Two of the cans, one filled with soil from each field, were treated with a proportionate amount of the chemical mixture used on the plots; the other two cans were left untreated and served as controls. In each can 10 alfalfa plants, 5 healthy and 5 having an early stage of dwarf, were set in such a way that healthy and diseased plants alternated. The object of this procedure was to determine whether the healthy plants would become diseased and whether the diseased plants would recover. The plants were set out on February 19, 1930.

Two plants in each of two cans, one treated and one control, and one plant in each of the other two cans failed to recover from the shock of transplanting; the other plants grew nicely. By October 21, 1930, all the diseased plants, except two in one of the control cans, had died; and two of the originally healthy plants, one in a treated can and one in a control can, had typical dwarf. The experiment was discontinued on July 3, 1931, when all the plants either had died from dwarf or had some stage of the disease. In no case did plants affected with dwarf outgrow the disease or healthy plants remain healthy. Although none of these experiments were conducted on a very large scale, the results are in agreement and are quite clear-cut; they indicate that ordinary fertilizers and other chemicals used have little or no effect on the susceptibility of alfalfa plants to the dwarf disease, and prove fairly conclusively that dwarf is due neither to an excess nor to a deficiency of any of these substances.

SUMMARY

Data are presented in this paper which indicate that dwarf, a recently described alfalfa disease whose cause is yet unknown, is largely responsible for the rapid dying of alfalfa plants in southern California.

Plots irrigated twice each month during the growing season and cut regularly were worthless for commercial hay production by the middle of the fourth year, whereas other plots similarly treated but allowed to remain without irrigation during the seed-growing period had fair stands at the end of the fourth year. The experimental evidence obtained has confirmed field observations that soil moisture exerts a marked effect on the severity of dwarf and consequently on the premature dying of alfalfa in southern California. Where soil moisture is kept sufficiently low, dwarf is not a factor in alfalfa culture. It hardly seems probable, however, that it will be commercially practicable to keep the soil moisture low enough to control dwarf and yet to maintain production at a desirable level.

Cutting alfalfa plants in the bud stage hastened the thinning of the stands to some extent, but not sufficiently to be of much commercial importance.

The development of dwarf in the plant is influenced to some extent by seasonal temperature, but its distribution is probably not limited by temperature to the regions in which it now exists.

Soil type does not appear to be much of a factor in the severity of dwarf. Neither manure, superphosphate, lime and sulphur, potassium, boron, manganese, strontium, bromine, titanium, iodine, aluminum, copper, or zinc were effective in controlling dwarf or in prolonging the life of the stand.

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EFFECT OF STEAM STERILIZATION ON SUSCEPTIBILITY OF WOOD TO BLUE-STAINING AND WOOD-DESTROYING FUNGI¹

By A. DALE CHAPMAN²

Formerly Agent, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

It is the ordinary procedure in experimental study of fungus action on wood to sterilize with steam the material to be inoculated, the treatments varying from a single autoclaving of 20 minutes at atmospheric pressure to repeated pressure treatments of 30 minutes each. A number of workers have suspected that such heating, especially the more severe pressure treatments, may change the chemical composition of the wood to a degree sufficient to make the wood an abnormal substratum for staining and wood-destroying fungi. This paper compares through the medium of strength tests the effects of different steam treatments on the susceptibility of certain species of southern pine to blue-staining and wood-destroying fungi.

BLUE-STAIN STUDY

METHODS AND MATERIALS

In any comparative study of the strength properties of wood, the selection and matching of test specimens are important. It

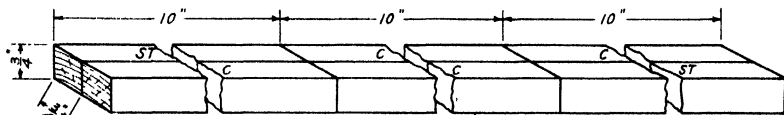


FIGURE 1.—A board sawn tangentially, from which six test specimens were taken. The specimens to be inoculated with stain fungi are marked ST, those kept as uninoculated controls are marked C.

was realized at the beginning of the study that since wood is a highly variable material, some knowledge of the comparative advantage of tangential and longitudinal matching should be had. In the first test, therefore, two control specimens, one tangentially and the other longitudinally adjacent, were taken for each specimen to be inoculated, as diagramed in figure 1. In this way the variability in strength properties of the control specimens alone could be determined and the different types of matching compared.

A statistical study of the variability of the strength values with the different types of matching was made, from which the advantage of matching specimens tangentially adjacent appeared to be only slightly greater than for specimens longitudinally adjacent. Both types of matching were therefore used in subsequent tests.

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² The writer wishes to express his appreciation to C. A. Richards and Carl Hartley, of the Division of Forest Pathology, and to T. R. C. Wilson, of the Forest Products Laboratory, for their valuable aid and criticism in the preparation of this paper.

Three species of wood, shortleaf pine, *Pinus echinata* Mill., loblolly pine, *P. taeda* L., and longleaf pine, *P. palustris* Mill., were used. All of the test specimens were cut from material taken at a height of from 8 to 16 feet in the tree, and carefully selected to get straight-grained wood of medium density and uniform growth. The bolts were split into sectors and the location of the specimens marked thereon in order to get true radial and tangential faces. All specimens were $\frac{3}{4}$ by $\frac{3}{4}$ by 10 inches in size.

The specimens were put into glass tubes at the bottom of which was placed distilled water to maintain a fairly high humidity and just enough broken glass to keep the stick from contact with the water. Two sterilization treatments were used, 30 minutes at atmospheric pressure and 5 minutes at 12 pounds' pressure. In two tests (nos. 4 and 5 of table 1) the specimens were sterilized outside of the tubes and transferred to sterile tubes inside a culture chamber. The same heat treatment was always given the matched controls except in the first two tests. In these tests the specimens to be inoculated were sterilized in glass tubes, whereas the control specimens were not tubed during sterilization. The latter group thus received the somewhat more severe heat treatment.

Some of the tests called for no steam treatment of the specimens. In these instances extreme care was exercised to keep contamination of specimens to a minimum. The planer and the saw were sterilized with mercuric-chloride solution and the specimens put immediately into sterile containers.

Inoculation consisted of spreading, inside a culture chamber, a heavy suspension of spores over the entire surface of each specimen. Two stain fungi were used, *Graphium rigidum* (Pers.) Sacc. (no. 198) and *Ceratostomella pilifera* (Fr.) Wint. (no. 185).

Complete coverage of each specimen with stain mycelium was usually obtained within 4 or 5 days. On the sticks that had not been sterilized this rapid development of mycelium kept mold contamination to practically nothing. Mold was observed to develop on only a few of the nonstained specimens; these were discarded.

The stain fungi were allowed to develop under optimum conditions of temperature and humidity for a period of 30 days before the specimens were tested. At the end of that time practically all of the specimens were stained throughout. Microscopic examination of the steamed and stained wood showed a greater abundance of fungus hyphae both in the ray cells and in the wood tracheids than is usually found in wood stained under lumber-yard conditions. Direct tracheid-wall penetration was also observed much more frequently than in normally stained wood, particularly with *Ceratostomella pilifera*. No marked change in color of the fungus hyphae from that found in unsteamed blue-stained wood was observed.

Three types of strength tests were made on most of the material—static bending, compression parallel to the grain, and toughness. The toughness test is a special test developed by the Forest Products Laboratory for testing small specimens. It is primarily a shock-resistance test, depending upon a combination of properties. All the specimens were tested in the green condition, in most cases well above the fiber saturation point.

RESULTS AND DISCUSSION

Table 1 presents the summarized results of the strength tests. Modulus of rupture and total work in bending were the values of static bending chosen for this summary, since they are of the most importance practically. Values for compression parallel to the grain are omitted. Values for toughness are given, since toughness is the strength quality most promptly affected by the action of fungi.

TABLE 1.—Effect of steam disinfection on capacity of stain fungi to weaken wood

Test no	Stain fungus	Species of wood	Heat treatment	Number of specimens	Average values for inoculated wood expressed as percentages of average values for noninoculated wood subjected to the same heat treatment			
					Specific gravity (oven-dry weight)	Static bending		Toughness
						Modulus of rupture	Total work in bending	
1	<i>Graphium rigidum</i>	<i>Pinus echinata</i>	None	16	103	99	89	75
	Do	do	30 minutes at 100° C.	16	99	90	82	76
	Do	do	5 minutes at 12 pounds' pressure	16	101	97	89	84
2	Do	<i>P. taeda</i>	None	25	98	96	92	77
	Do	do	30 minutes at 100° C.	25	104	96	67	63
3	Do	<i>P. palustris</i>	None	12	101	104	84	82
	Do	do	30 minutes at 100° C.	12	101	86	30	33
4	<i>Ceratostomella pilifera</i>	do	None	12	100	100	49	57
5	Do	do	30 minutes at 100° C.	12	100	100	37	38
	Do	do	None	18	99	97	67	60
	Do	do	30 minutes at 100° C.	18	98	101	52	44
Summation ^a			(None)	83	99.5	98.4	77.7	70.2
			30 minutes at 100° C.	83	100.5	94.8	54.2	50.5

^a The summation values used are the totals of the absolute strength values for the inoculated specimens expressed as percentages of the totals for the uninoculated

Table 1 affords a comparison between steamed and unsteamed wood with respect to the effects of stain fungi on strength values. Here the average strength values for the inoculated unsteamed wood are expressed as percentage ratios of the values for the natural uninoculated unsteamed wood, and the average values for the inoculated steamed wood expressed as percentage ratios of the average values for uninoculated steamed wood. In other words, the factor of the direct effect of heat on mechanical properties, which, incidentally, appears to have been abnormally high in these experiments, is eliminated, and the table thus furnishes a direct comparison of the effect of stain fungi on wood with and without steam treatment.

Specific gravity was not perceptibly affected by either fungus. *Ceratostomella pilifera* had no apparent effect on modulus of rupture, but the *Graphium rigidum* sticks were lower in this value than the uninoculated in the heated wood in all three of the experiments; the decreases were so small that both their mathematical significance and their practical importance are doubtful. A very different result was obtained with the toughness test, and also with the static bending total work values, which are reflections of toughness more than of

strength. In these values the fungi caused an average reduction of approximately one fourth in unheated wood and nearly one half in wood that had been steamed. The reduction was somewhat greater in toughness than in total work, and the *Ceratostomella* seemed to have more effect on these values than the *Graphium*. The apparent difference between the fungi may have been increased by the fact that in test 1 some of the *Graphium* sticks were not fully infected; in any case a larger number of tests and tests with other strains of the fungi would be needed to establish the greater effectiveness of the *Ceratostomella*.

The results indicate that stain fungi may affect very appreciably certain of the mechanical properties of wood, and that the steam-sterilization treatments commonly used in experiments with fungi on wood decidedly increase the susceptibility of the wood to deterioration by stain fungi. It would not be safe to conclude from them that wood given a commercial heat treatment, such as kiln drying or steaming, is much more subject to deterioration by fungi than is natural unheated wood, or that the occurrence of stain in lumber would necessarily mean a pronounced loss in toughness of the wood. In this experimental work the conditions for stain development were optimum, and these conditions maintained for a period of 30 days were probably more conducive to deterioration by stain fungi than those ordinarily present in the lumberyard, where pronounced variations in humidity and temperature are usually encountered.

The chief value, then, of the results of these few tests lies not in respect to the effect of heat treatment of wood as lumber, but in the fact that they are significant to those studying the effect of fungi on wood. Conclusions that may be drawn from experiments in which the wood has been steam sterilized may be subject to considerable error unless due consideration of its effect is given in the interpretation of results. It would seem necessary to qualify the acceptance of some of the past experimental work that has been done with wood disinfected by heat.

Table 2 gives an idea of the variation, or change in the observed average, for the different treatments for the three tree species. A comparison of the coefficients of variation for unheated wood indicates that staining, as might be expected, is a highly variable factor and increased the variability of the wood in toughness strength; but in heated wood there was no indication of increased variability due to inoculation in either *Pinus echinata* or *P. taeda*. If any conclusion be drawn on so small a basis of evidence, it is suggested that some factor in the unheated sticks of all three pine species causes them to differ in their susceptibility to attack by the fungi used; but that in two of these species heat treatment by destroying inhibiting factors or increasing the digestibility to fungi of the cell walls has made the different sticks more nearly equal in susceptibility to deterioration than before heating.

TABLE 2.—Effect of treatment on standard deviation and coefficient of variation in toughness test

Treatment	Species of wood	Number of specimens	Mean	Standard deviation	Coefficient of variation
Natural, not steamed, not inoculated....	<i>Pinus echinata</i> ...	16	280	57.2	20.4
	<i>Pinus taeda</i>	25	508	65.1	12.9
	<i>Pinus palustris</i> ..	24	515	77.6	15.1
Not steamed, inoculated.....	<i>Pinus echinata</i>	16	204	87.3	42.8
	<i>Pinus taeda</i>	25	400	96.2	24.1
	<i>Pinus palustris</i>	24	390	87.2	22.3
Steamed 30 minutes at 100° C., inoculated.	<i>Pinus echinata</i>	16	184	34.4	18.8
	<i>Pinus taeda</i>	25	321	35.6	11.1
	<i>Pinus palustris</i>	24	205	77.5	38.0

DECAY STUDY

METHODS AND MATERIALS

Decay fungi were employed only in a single experiment. The material was green loblolly pine sapwood (*Pinus taeda*), cut into longitudinally aligned specimens $\frac{3}{4}$ by $\frac{3}{4}$ by 4 inches in size, with 2-inch samples for moisture determination distributed alternately between them. The moisture content of each 4-inch test specimen was assumed to be the same as the average of its two adjacent 2-inch samples, and its oven-dry weight was thus computed from its green weight. Alternate 4-inch test specimens were given one of two steam treatments—30 minutes autoclaving at atmospheric pressure, or 5 minutes at 12 pounds—and were compared with the intermediate control specimens that had been unheated. The unheated specimens were sawed out under as aseptic conditions as possible, and placed immediately on fungus mats in Kolle flasks; their green weight was taken as the average of the two adjacent specimens of the same size. Half of the specimens were placed on mycelial mats of *Poria incrassata* (Berk. and Curt.) Burt., and half on mats of *Lentinus lepideus* Fr., both of which decay pine at about the same rate. The percentage loss in weight was determined at the end of 30 days for part of the specimens, and at the end of 70 days for the remainder.

RESULTS AND DISCUSSION

As was expected, at the time of final inspection a number of the specimens (8 of the 18 unheated specimens) were contaminated with molds, which in all probability tend to inhibit growth of the wood destroyers. All the visibly molded specimens except one which was very lightly molded were discarded. The discarding of the molded specimens may have involved loss of the most susceptible wood. The presence of contamination also throws doubt on the accuracy of the results and prevents clear-cut conclusions being drawn. The results are presented, however, in the belief that they are indicative. Further work is necessary.

Table 3 indicates that after the first 30 days decay was relatively slower for the natural unheated specimens and that the 5-minute pressure treatment predisposed the wood to decay to a greater degree than the 30-minute treatment at atmospheric pressure. Roughly, there is a ratio of 3:2:1 for the amount of decay at the end of 70 days for the three types of treatment, 5 minutes at 12 pounds' pressure,

30 minutes at atmospheric pressure, and no steaming; all of which indicates that steam sterilization, especially with pressure and resultant higher temperatures, makes the wood a more suitable substratum for these wood-destroying fungi after the fungus has passed the earliest invasion stage.

TABLE 3.-*Percentage loss in weight of wood specimens due to decay*

Sterilization treatment	Period of decay, 30 days		Period of decay, 70 days	
	Speci- mens	Loss in weight	Speci- mens	Loss in weight
	<i>Number</i>	<i>Percent</i>	<i>Number</i>	<i>Percent</i>
5 minutes at 12 pounds' pressure.....	15	2.14	21	19.95
30 minutes at atmospheric pressure.....	13	3.13	11	11.49
None.....	11	2.16	20	6.77

WOOD STERILIZATION METHODS

It appears that other sterilization methods must be employed if the wood used in laboratory experiments is to simulate wood in the natural state. A number of methods have been suggested and tried, most of them chemical, involving the use of volatile toxic materials, but with little assurance that their application does not change the composition of certain constituents of the wood.

SUMMARY

From the results of the experiments with heated and unheated pine wood on the effect (1) of staining fungi on strength properties and (2) of wood-destroying fungi on the loss in weight of wood, it appears that even the relatively mild treatments ordinarily used in disinfecting blocks for wood-decay experiments may so change the wood as to make it a more congenial substratum than normal unheated wood for at least some of the wood-deteriorating fungi. Consequently, any conclusions that may be drawn from experiments in which the wood has been steam sterilized are subject to considerable error, unless due consideration is given to the effect of such sterilization in the interpretation of results. In view of the evidence submitted here, it seems necessary to qualify the acceptance of some of the past experimental work that has been done with wood disinfected by heat.

SOME PHYSIOLOGICAL EFFECTS OF ULTRAVIOLET RADIATION ON HONEYBEES¹

By LLOYD M. BERTHOLF²

Formerly field assistant in apiculture, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

The beneficial effects of ultraviolet light on higher animals such as chickens, rats, and human beings are now well known. It has been used with great success in the treatment of such diseases of man as tuberculosis of the skin, bones, or viscera, skin diseases of various kinds, rickets, and tetany. Not so well known, perhaps, are the harmful effects of ultraviolet light on various organisms. Bacteria, for example, are readily killed by the so-called "middle ultraviolet", that is, by radiations of wave lengths between 210 and 297 $m\mu$ ³; plants are rather generally harmed by wave lengths shorter than 290 $m\mu$ ⁴; and animals, including man, may suffer from serious burns or internal disorders from it under certain conditions.

The possibility of finding in ultraviolet light a source of benefit to bees, and consequently to the beekeeping industry, has led to a few investigations on the physiological effect of these rays on honeybees. Probably the first was that performed by two Hungarian workers, Stitz and Beyer, in 1927.⁵ These workers irradiated one colony of bees and kept another, as nearly identical as possible, as a control. They describe the method and results of their experiment in part as follows:

* * * Naturally the main experiment was carried on for several weeks, always with intensive irradiation. The combs with the bees were irradiated individually and were always checked against the other nonirradiated colony, which was equally strong and handled otherwise in exactly the same way. The result was startling. The days after the irradiation one recognized at once, "among 100 colonies", the irradiated ones by their heightened activity and their active coming and going. Moreover, metabolism was decidedly stimulated. The irradiated colonies built about 1½ times as many new cells as the controls, but most of all was to be noticed the action of ultraviolet rays on the development of the larvae. Their development was shortened by two days. When the irradiation was overdone—as took place in the last test of the main experiment—the bees came out of their cells before they were completely developed, so that their abdomens were still entirely white. (Naturally, these individuals died after a short time.) * * * One can therefore conclude that the influence of ultraviolet rays is favorable to the different life phenomena of bees.

The only other work done along this line, so far as the writer is aware, is that of Muth and Balinkin^{6 7} in the United States. This

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² The writer wishes to express his gratitude to Jas. I. Hambleton, in charge of the Division of Bee Culture, Bureau of Entomology, U.S. Department of Agriculture, where the present investigation was made, for facilities granted and for many useful suggestions, and to Rockwood Nusbaum, commercial beekeeper of Union Bridge, Md., with whose cooperation some of the experiments on queens were carried out.

³ ELLIS, C., and WELLS, A. A. THE CHEMICAL ACTION OF ULTRAVIOLET RAYS. 362 p., illus. New York. 1925.

⁴ ARTHUR, J. M., and NEWELL, J. M. THE KILLING OF PLANT TISSUE AND THE INACTIVATION OF TOBACCO MOSAIC VIRUS BY ULTRAVIOLET RADIATION. Amer. Jour. Bot. 16: 338-353, illus. 1929.

⁵ STITZ, J., and BEYER, M. DIE BIOLOGISCHE WIRKUNG DER ULTRAVIOLETTEN STRAHLEN—AUF DIE BIENEN. Arch. Bienenkunde 8: 286-288. 1927.

⁶ MUTH, C. F. ULTRA VIOLET RAY AND QUEENS. Beekeepers Item 11(5): 147-148. 1927.

⁷ ———. ULTRA VIOLET RAYED QUEENS. Iowa State Apiarist Rpt. 1928: 56-64, illus. 1928.

work was started in 1926 and apparently has been continued ever since. These men claim to have noted such decidedly beneficial effects on the queens as a result of the treatment that they have applied for a patent on their process and have placed irradiated queen bees on the market, selling them for a higher price than ordinary queens. According to the meager data which they published,⁸ they used first a carbon arc and later a quartz mercury-vapor lamp, filtered the rays through a glass filter which "shut out the wave lengths less than 2,700 angstrom-units", and, in the only one of their successful experiments for which the length of exposure is stated, gave the queens a 15-second exposure on the first day, no exposure on the second day, a 30-second exposure on the third day, no exposure on the fourth day, and a 90-second exposure on the fifth day. Neither the distance from the lamp nor the size of the lamp is stated.⁹

With this experiment there was a steady increase in egg-laying on all of the treated colonies until at the sixth week there was a 30 percent increase in egg-laying over and above that of normal.

They also found that the progeny of the treated queens were much more gentle than ordinary colonies, and that even Cyprian bees, which are notorious for their bad temper, were made gentle by having come from a treated queen.

Muth and Balinkin⁸ also treated some queen larvae with ultraviolet rays.

We treated a young queen cell all during its period of development, keeping the frame which contained this queen cell in our office. * * * When this queen emerged * * * she was without doubt the largest queen that my father, several beekeepers who have seen her, and myself have ever witnessed in our lives.

These reports are very interesting, and, if they are confirmed by subsequent work, treatment of bees with ultraviolet rays ought to have both a theoretical and a practical importance—theoretical in that it may lead to a better understanding of the physiological action of ultraviolet light, and practical in that it may enable us to increase the honey crop and perhaps eventually to apply the results to other insects or other animals.

To extend the investigations already made and to check the results more carefully, the experiments described in this paper were performed. The effect of ultraviolet rays was tested first on adult workers and then on worker larvae, since such material is plentiful, and finally the knowledge thus gained was applied to the more limited supply of queens, both adults and larvae.

EFFECT OF ULTRAVIOLET RADIATION ON THE LONGEVITY OF ADULT WORKERS

Of the various criteria for ascertaining the physiological effect of ultraviolet radiation on an animal, longevity is one of the easiest to measure and is probably as good an indication of the total physiological effects as any. It was therefore chosen in this series of experiments.

In the first experiment 1-day-old adult workers were used, but they lived for such a short time that in subsequent experiments slightly

⁸ MUTH, C. F. See footnote 7.

⁹ Since this report was written the patent applied for has been issued (U.S. Patent 1,868,042; issued July 19, 1932). In the application for patent (Mar. 23, 1929) some additional data are given as to the method of treatment. The queens are placed "around 39 inches" from the lamp and exposures of "around one minute, two minutes and five minutes", respectively, are made on 3 successive days.

older bees were used. These were obtained by shaking the bees from a frame onto the ground and picking up those that did not fly away. This assured one of getting fairly young bees (ordinarily less than 2 weeks old), since the older ones, the field bees, flew away at once. As the bees were picked up they were put into a specially designed cage, 50 bees to a cage.

The bottom and the top of the cage (fig. 1) were made of heavy copper-wire screen, and the sides and ends of wood. Over the vestibule at the front of the cage was a sliding cover provided with a hole into which was inserted the open end of a large test tube containing queen-cage candy. The cover could be taken out for cleaning by sliding it forward, or it could be slid back far enough to permit removal of the dead bees with forceps after they had been shaken down to the

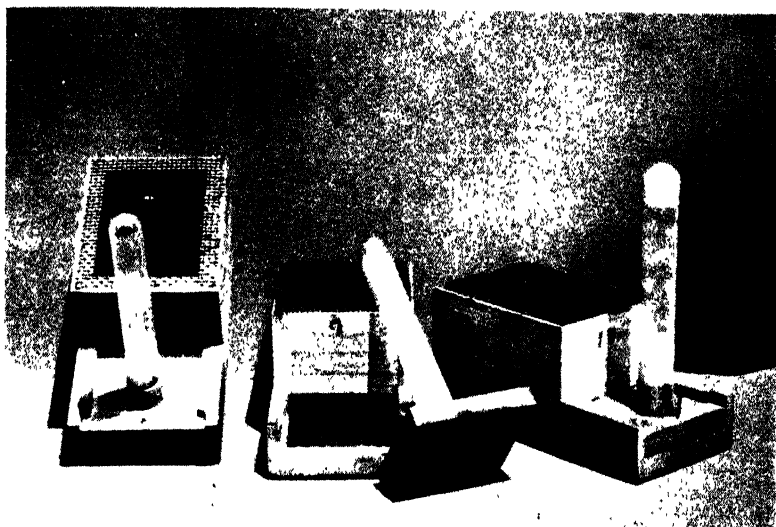


FIGURE 1 --Three views of the cage used for keeping adult worker bees in confinement and for exposing them to light. The glass tube contains queen-cage candy for food.

front. The cages, when new, were soaked in hot paraffin; hence it was easy to clean them with soap and water before starting an experiment. During an experiment they were kept lying on one side, thus holding the candy tube in a horizontal position so that the bees, after becoming feeble, could get food easily.

The irradiation was done through the bottom of the cages. A quartz mercury-vapor lamp (Uviarc), 220-volt size (fig. 2), was used and was run at about 4 amperes. It was provided with a hood, which also served as a reflector. At the front of the hood was a cover having an opening 6 inches square, over which any desired filter could be placed. During the course of the investigation three glass filters—Pyrex, Corex D, and Corex A red-purple—were used. The quality and quantity of light transmitted by these filters, given in table 1, were measured by R. Stair, of the United States Bureau of Standards. The intensity of irradiation 31.5 inches from the lamp was sufficient to produce a decided erythema on untanned skin in about 1 minute.

TABLE 1.—*The quality and quantity of radiant energy emitted by the quartz mercury-vapor lamp and transmitted by three glass filters*

Filter used	Lower limit of spectrum emitted or transmitted	Upper limit of spectrum emitted or transmitted	Relative energy emitted or transmitted
	<i>Millimicrons</i>	<i>Millimicrons</i>	<i>Percent</i>
Open lamp, no filter	185	1,400+	100.0
Corex D	297	1,400+	24.5
Pyrex	313	1,400+	22.6
Corex A red-purple	265	405	4.7

The cages of bees were examined daily except Sundays, and all dead bees were counted and removed. Dead bees were assumed to

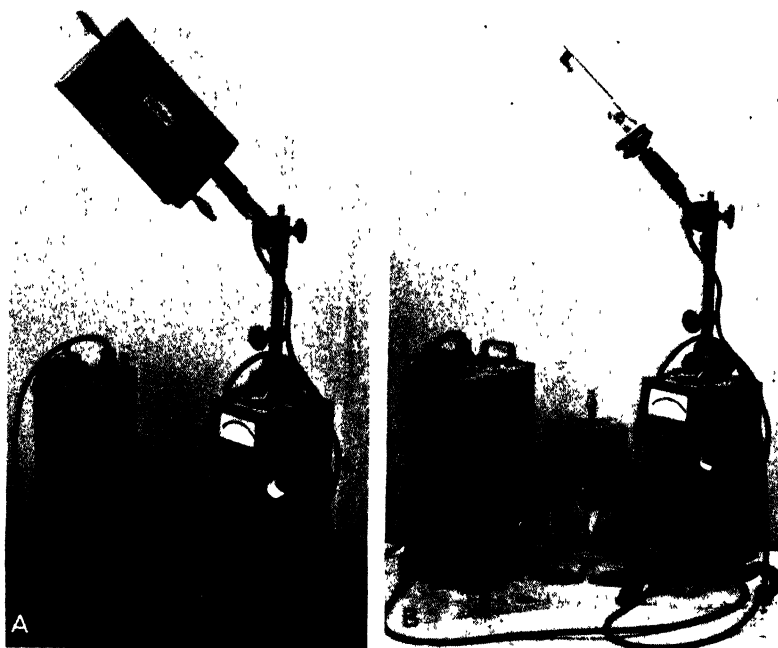


FIGURE 2.—Two views of the quartz mercury-vapor lamp and accessories used in these experiments: A, With lamp house and filter in place; B, with lamp house and filter removed. The large apparatus at the left is a rectifier for making direct current from alternating current.

have died midway between the time at which they were found dead and the preceding examination of the cage, and the mean longevity for the entire 50 bees in each cage was computed on this assumption. The probable error of the mean was computed according to Peters' formula

$$r_0 = \frac{0.8453 \Sigma v}{n \sqrt{n-1}}$$

where v = the variation of the length of life of each bee in the cage from the mean for the whole cage, and n = the number of bees in the cage.

Except while the bees were being counted or irradiated, the cages were kept in a dark room maintained at about 21° C. (70° F.). Six experiments were performed in the manner outlined above.

EFFECT OF LIGHT FROM OPEN LAMP

The effect of the light from the open lamp in exposures of various length was first tested. The bees were all very young, 1 day old or less. The cages were placed 12 to 14 inches from the lamp and given exposures varying from a single exposure of 30 minutes down to a 10-second exposure on 3 different days. The results are presented in table 2. It will be noted that in no case did the bees in the treated lots live as long as the untreated controls. The fact that these bees were very young, however, and that the distance from the lamp was so short that there was a considerable radiation of heat (the exact temperature was not taken) may have had more influence on longevity than had the light.

TABLE 2.—*The longevity of adult honeybees, 1 day old or less at the start of the experiment, when exposed at a distance of 12 to 14 inches for various lengths of time to the unfiltered light from a quartz mercury-vapor lamp, as compared with the longevity of untreated bees*

Cage no.	Treatment	Maximum longevity	Average longevity
		<i>Days</i>	<i>Days</i>
5	Control, untreated.....	14	6.74±0.20
6	30 minutes first day only ^a	10	3.99±.20
10	15 minutes first and second days only.....	6	2.24±.05
11	5 minutes daily ^b	6	3.38±.13
12	1 minute daily ^b	11	5.87±.16
15	15 seconds daily ^b	13	5.65±.23
17	5 seconds daily ^b	7	3.29±.11
22	30 seconds first, second, and fourth days each.....	12	6.22±.20
23	10 seconds first, second, and fourth days each.....	9	3.22±.17
25	30 seconds first day, 1 minute second day, 1½ minutes fourth day.....	10	4.32±.18

^a Temperature at cage at end of this period was 43° C. (109° F.).

^b As long as any bees in the cage remained alive.

TABLE 3.—*The longevity of adult bees, about 1 week old at the start of the experiment, when exposed at a distance of 31.5 inches for various lengths of time to the unfiltered light from a quartz mercury-vapor lamp, as compared with the longevity of untreated bees*

Cage no.	Treatment ^a	Maximum longevity	Average longevity
		<i>Days</i>	<i>Days</i>
4	Control, untreated.....	21	14.72±0.26
8	30 minutes.....	14	7.59±.32
10	15 minutes.....	14	7.70±.32
11	5 minutes.....	23	7.30±.37
13	1 minute.....	25	17.62±.47
18	½ minute.....	28	18.45±.36

^a All treatments were on alternate days as long as any bees remained alive in the cage

A second experiment was therefore performed in essentially the same way except that slightly older bees, about 1 week old, were used, the distance from the lamp was increased to 31.5 inches, and exposures were made on alternate days as long as any of the bees in the cages remained alive. This time the highest temperature during exposure was 35° C. (95° F.) as compared with 30° C. (86° F.) prevailing in the room. This difference was probably not enough to affect longevity appreciably. The results are presented in table 3.

In this experiment exposures of 30, 15, and 5 minutes on alternate days were all decidedly harmful to the bees, but exposures of 1 minute and one half minute on alternate days were beneficial.

EFFECT OF THE ULTRAVIOLET PORTION OF THE LIGHT

The result of this experiment suggested the question as to whether the benefit apparently caused by the light was due to its short-wave, ultraviolet portion or to the longer wave lengths. To answer this question it was decided to use a filter for the light which would cut off the longer radiations and transmit only the ultraviolet. This was easily done by using the Corex A red-purple filter, which transmits only wave lengths between 265 and 405 $m\mu$ (table 1). The exposures were made as in the preceding experiment, that is, on alternate days and at a distance of 31.5 inches from the lamp. The lengths of exposure were 1, one half, and one fourth minute each, since the shorter periods had seemed to be beneficial in the previous experiment.

The results are presented in table 4. None of the treated bees lived longer than the controls, and the only ones that lived as long as the controls were in the cage exposed one fourth minute. This indicates that, if any of the light from the quartz mercury-vapor lamp is beneficial, it must be either the light of a wave length longer than 405 $m\mu$ or of one shorter than 265 $m\mu$.

TABLE 4.—*The longevity of adult bees when exposed at a distance of 31.5 inches for various lengths of time to the light from a quartz mercury-vapor lamp filtered through a Corex A red-purple filter, as compared with the longevity of untreated bees*

Cage no	Treatment *	Maximum longevity	Average longevity
		<i>Days</i>	<i>Days</i>
6	Control, untreated.....	16	9.79±0.46
12	1 minute.....	19	7.22±.66
17	$\frac{1}{2}$ minute.....	19	5.89±.52
23	$\frac{1}{4}$ minute.....	16	9.74±.54

* All treatments were on alternate days as long as any bees remained alive in the cage.

EFFECT OF LIGHT OF VARIOUS WAVE LENGTHS

In order to determine whether it is the very short wave lengths or those longer than 405 $m\mu$ that cause the apparent increase in longevity, exposures were made to light filtered through Corex D, Pyrex, and Corex A red-purple filters, respectively, in all treated cages except the one irradiated with the open lamp for comparison. As indicated in table 1, Corex D and Pyrex cut off wave lengths shorter than 297 and 313 $m\mu$, respectively, but transmit the longer waves freely. The length of each exposure was either 1 minute or one half minute, and exposures were made on alternate days and at a distance of 31.5 inches as before. The results are presented in table 5. All the irradiated bees lived as long as the controls, but none significantly longer except those irradiated by unfiltered light, which outlived the controls by an average of about 4 days. In this lot also the maximum longevity was remarkably great, one bee living 35 days—14 days longer than the best among the controls.

TABLE 5.—*The longevity of adult bees when exposed at a distance of 31.5 inches for various lengths of time to the light from a quartz mercury-vapor lamp, both unfiltered and filtered through three specified filters, as compared with the longevity of untreated bees*

Cage no.	Treatment ^a	Maximum longevity	Average longevity
		<i>Days</i>	<i>Days</i>
4	Control, untreated.....	21	14.16±0.30
5	Open lamp, ½ minute.....	35	18.65±.71
6	Corex D filter, 1 minute.....	24	14.26±.56
8	Corex D filter, ½ minute.....	23	14.25±.29
10	Pyrex filter, 1 minute.....	25	14.80±.55
15	Pyrex filter, ½ minute.....	27	16.00±.38
22	Corex A filter, 1 minute.....	23	15.38±.41
23	Corex A filter, ½ minute.....	24	14.74±.46

^a All treatments were on alternate days as long as any bees remained alive in the cage.

Since the increased longevity under the unfiltered light is not due to radiations between 265 and 405 $m\mu$, as shown by the results in cages 6 and 8 (also indicated in the previous experiment, table 4), and since it is evidently not due to wave lengths longer than 297 $m\mu$ (cages 22 and 23) or 313 $m\mu$ (cages 10 and 15), the conclusion is reached that, if it is due to any part of the light, it must be due to wave lengths shorter than 265 $m\mu$. This conclusion is unexpected because neither in the experiments on other animals nor in those on plants has it been found, so far as the writer is aware, that radiations of wave length shorter than about 290 $m\mu$ are beneficial. These rays are not present in sunlight and are commonly called the "abiotic" or lethal rays.¹⁰

EFFECT OF EXPOSURE TO LIGHT OF VARIOUS WAVE LENGTHS BUT OF CONSTANT QUANTITY

There is another possible explanation of these results, however. Perhaps the increased longevity under radiation from the open lamp (table 5) is due, not to the quality of its light, but only to the quantity. With all cages placed 31.5 inches from the lamp, the one irradiated without a filter received much more energy than the others, and this may have been the optimum for greatest benefit to the bees. In order to test this explanation an experiment was performed in which the cages were placed at such distances from the lamp that the quantity of energy coming to each was the same, as ascertained by the transmission data given in table 1.

A rack made for this purpose was placed before the lamp, as shown in figure 3. Each cage was held on the inclined board by nails driven at such distances that when irradiated with light from the open lamp the center of the cage was 72.6 inches from the lamp tube, and when irradiated with light filtered through the Pyrex, Corex D, and Corex A glasses, at 34.5, 35.9, and 15.7 inches, respectively. All the cages except the controls were given 1½ hours' total exposure, divided as indicated in table 6. As the tests were made on duplicate cages, there were 100 bees in each lot instead of 50 as previously.

The results, presented in table 6, show that no lot lived significantly longer than any other. It is therefore fairly certain that the increased

¹⁰ ELLIS, C., and WELLS, A. A. See footnote 3,

longevity observed previously in some of the cages was not due to the quality of the light received in those cages. It may be possible that the bees in those cages received a certain optimum quantity of energy, or that the increased longevity observed was not a real increase but

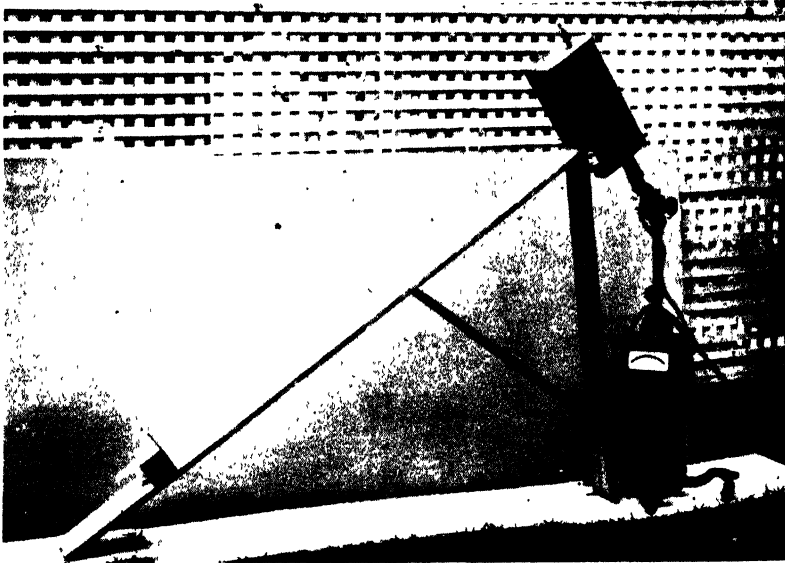


FIGURE 3.—Rack used for holding the cages of adult bees while exposing them to the light, shown in place before the lamp. The cage rests on nails driven part way into the board.

was wholly within the limits of experimental error. The writer is inclined toward the latter conclusion.

TABLE 6.—*Longevity of adult bees when exposed on the first nine consecutive days for 1, 2, 3, 4, 5, 10, 15, 20, and 30 minutes, respectively, to the light from a quartz mercury-vapor lamp, both unfiltered and filtered through three different filters at such distances that the energy coming to each cage was the same, as compared with the longevity of untreated bees*

Cage nos.	Treatment	Maximum longevity	Average longevity
		<i>Days</i>	<i>Days</i>
31 and 32	Controls, untreated.....	18	10 66±0. 15
9 and 13	Open lamp at 72.6 inches.....	18	10 96±. 22
14 and 15	Pyrex filter at 34.5 inches.....	17	10 98±. 26
18 and 21	Correx D filter at 35.9 inches.....	18	11 23±. 31
25 and 30	Correx A filter at 15.7 inches.....	17	11. 05±. 24

EFFECT OF IRRADIATION OF THE BEES' FOOD

There remains, however, still another possible explanation. Perhaps the light does not affect the bees directly, but only their food, and irregularities in the shading of the tube of food by the bees' bodies during irradiation may account for the discrepancies in the results of the different experiments. To test this explanation the food (queen-cage candy) for one cage of bees was spread out in a thin

layer and irradiated for 1 hour at 31.5 inches from the open lamp before being placed in the tube. Bees fed on this food were compared with others fed on nonirradiated food. The results, given in table 7, show that the controls lived slightly longer than the bees fed on irradiated food. Evidently, then, the irradiation of the food has no significant influence on the longevity of bees.

TABLE 7.—*The longevity of bees fed on candy that had been irradiated by the unfiltered light from a quartz mercury-vapor lamp, as compared with the longevity of bees fed on nonirradiated food*

Cage no.	Treatment	Maximum longevity	Average longevity
		Days	Days
6	Control, food untreated.....	16	9.79±0.46
25	Food irradiated.....	19	7.68±.52

SUMMARY OF RESULTS ON ADULT WORKER BEES

The general result of these experiments is, therefore, that direct irradiation of adult worker bees, whether by the total light from a quartz mercury-vapor lamp, or by the portion of the light above 313 $m\mu$ wave length, or by the portion above 297 $m\mu$, or by the portion between 265 and 405 $m\mu$, within the fairly wide limits of dosage used, was ineffective in increasing the length of life of bees in confinement but decreased the length of life of bees exposed for longer intervals, and that irradiation of the food only was likewise ineffective in increasing longevity.

SOME EFFECTS OF ULTRAVIOLET LIGHT ON WORKER LARVAE

In view of the claim by Stitz and Beyer¹¹ that the developmental period is shortened by irradiating the brood with light from the quartz mercury-vapor lamp, and the probability that larvae, with their thin sclerotic covering, are much more susceptible than adults to influence by these rays, it became especially interesting to investigate the effect of ultraviolet light on the larvae.

Broods at nearly every age were used in one or another of the experiments, but particularly larvae 2 to 3 days old. They were irradiated in the combs by taking the frame containing them out of the hive, shaking off all adult bees, and holding the frame in front of the lamp. The lamp used in the first few experiments was a carbon arc with Therapeutic C carbons (a carbon impregnated with iron, nickel, and aluminum, the arc of which is especially rich in ultraviolet light), 8 mm in diameter, and was run at 5½ amperes and 35 volts alternating current. For the other experiments the quartz mercury-vapor lamp already described was employed. The filters used were those given in table 1.

Usually only a single exposure was given, but sometimes a second exposure was given 1 day after the first. Opaque screens of various kinds were placed over portions of the brood area to shield these larvae from the light so that such areas could be used as controls. At the time of irradiation the larvae in each area were counted.

¹¹ STITZ, J., and BEYER, M. See footnote 5.

Examinations were then made at intervals of from one to several days to note the effect, and finally in some experiments upon emergence to note whether the irradiated brood emerged sooner than the controls. In one experiment the emerging bees were weighed to see if they differed in size from the controls. The details of each experiment are best described in connection with the results.

DOSAGE OF LIGHT LETHAL TO LARVAE

The first experiment was exploratory in nature, designed to see how great a dosage of the light from the carbon arc was necessary to kill the larvae. Five holes, each 2 inches square, were cut in an opaque screen the size of an ordinary frame in a beehive. This screen was placed over a frame of brood, the larvae of which were $1\frac{1}{2}$ to $2\frac{1}{2}$ days old, and the areas exposed through the holes were irradiated for one fourth, one half, 1, 3, and 10 minutes, respectively, at 7.8 inches from the arc. The larvae in each irradiated area were counted at the beginning of the experiment and again after 1, 2, 3, 4, 5, 7, 9, 10, and 12 days.

The results are shown in table 8. It will be noted that at all exposures except one fourth minute virtually all the brood was killed within 12 days, and that even at this exposure 80 percent were killed; that is, 80 percent of the brood were removed from the cells by the bees, and it is therefore assumed that they were killed by the light. The total effect is shown strikingly in the photographs of the frame both with and without the screen (fig. 4). Although no count of the larvae present on succeeding days was made on the nonirradiated areas, the photograph of the entire frame (fig. 4, A) shows that at least 90 percent of the larvae there survived.

TABLE 8.—*The lethal effect on brood, $1\frac{1}{2}$ to $2\frac{1}{2}$ days after hatching, of the unfiltered light from an electric carbon arc using Therapeutic C carbons, operated at $5\frac{1}{2}$ amperes, 35 volts alternating current, and placed 7.8 inches from the comb*

Area irradiated	Duration of irradiation	Larvae present at beginning	Percentage of original number alive after indicated number of days								
			1	2	3	4	5	7	9	10	12
	Minutes	Number									
a	$\frac{1}{4}$	85	100	72	41	41	41	38	27	27	20
b	$\frac{1}{2}$	95	100	47	38	35	33	4	3	3	1
c	1	115	99	9	6	4	3	1	1	1	1
d	3	115	94	48	33	27	20	6	3	3	1
e	10	115	3	3	3	3	3	2	2	2	1

* Most cells containing larvae were capped over on this day.

^b Photographs taken on this day are shown in figure 4

^c The higher percentages in this area than in c are probably due to the fact that these larvae were slightly older than those in c.

In another experiment the effect of a 5-second exposure to the same light at a distance of 7.8 inches, on broods of different ages, was tested. The eggs, larvae just hatched, and larvae 1 day old were all killed by this treatment, but larvae 2 days old were not.

The surprising thing shown by these two experiments was the remarkably small resistance of brood to the light from this carbon arc. It was thought that perhaps this light was too rich in ultra-violet or in infrared rays, or for some other reason could not be com-

pared with that from the mercury-vapor lamp used with such success by Stitz and Beyer. Hence, the quartz mercury-vapor lamp (Uviarc) was brought into use. After some preliminary tests the following experiment was made:

Larvae of definite ages were obtained by confining a queen bee overnight in a part of the hive in which there was only a single empty comb. During the night she laid eggs in a considerable area on both sides of this comb. In the morning she was moved to another part of

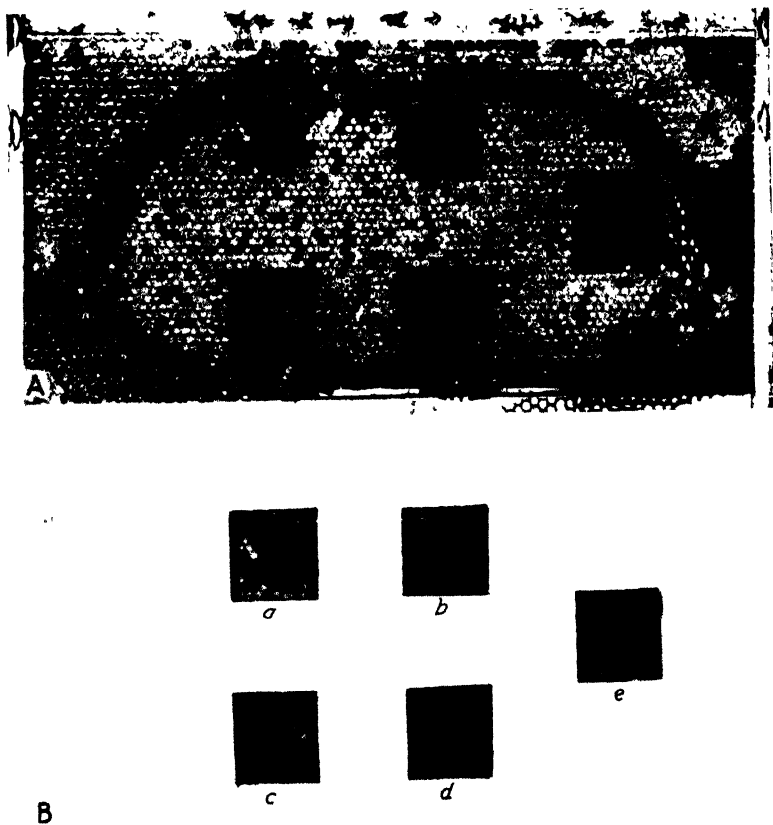


FIGURE 4.—Photographs of a frame of sealed brood in which various areas had been exposed to ultraviolet light while the larvae were $1\frac{1}{2}$ to $2\frac{1}{2}$ days old: A, The frame without the screen; B, the frame covered by the screen containing the holes through which exposures were made. The holes in the screen are lettered *a* to *e* as in table 8.

the hive, where she could not get to this comb again, and the eggs in the comb were allowed to develop. When the resulting larvae were $3\frac{1}{2}$ days old ($6\frac{1}{2}$ days after the eggs were laid), the comb was taken from the hive, one side was covered with the screen already described (fig. 4, B), and four of the areas lying under the holes in the screen were given irradiations of 5, 10, 15, and 20 seconds, respectively, 31.5 inches from the mercury-vapor lamp. After the larvae in each irradiated area were counted, the comb was replaced in the hive. The next day the other side, with larvae now $4\frac{1}{2}$ days old, was given the

same treatment. The irradiated areas were examined 1, 2, 5, 9, and 12 days after their irradiation.

The results presented in table 9 show that, although there was considerable variation in the death rate, yet after 12 days all the irradiated larvae, except 70 percent of those $4\frac{1}{2}$ days old that were given the 5-second treatment, were dead. Apparently, then, the light from the mercury-vapor lamp is just as harmful to the brood as is that from the carbon arc. Observations as to the time of emergence of these $4\frac{1}{2}$ -day-old larvae which survived the 5-second treatment gave no evidence that they emerged as adult bees any sooner or later than did bees in nonirradiated areas of the same comb.

TABLE 9.—*The lethal effect on brood, $3\frac{1}{2}$ and $4\frac{1}{2}$ days after hatching, of the unfiltered light from a quartz mercury-vapor lamp run at 4 amperes and 140 volts direct current and placed 31.5 inches from the comb*

Age of larvae (days)	Area of comb irradiated ^a	Duration of exposure	Larvae treated	Percentage of original number alive after indicated number of days				
				1	2	5	9	12
$3\frac{1}{2}$		Seconds	Number					
	a	5	17	12	1	0	0	0
	b	10	28	61	54	21	0	0
	c	15	27	33	19	8	0	0
	d	20	25	0	0	0	0	0
$4\frac{1}{2}$	a ¹	5	27	100	93	90	70	70
	b ¹	10	10	100	90	80	10	0
	c ¹	15	17	47	35	1	0	0
	d ¹	20	32	94	88	66	0	0

^a The areas designated as a¹, b¹, c¹, and d¹ were on the opposite side of the comb to the areas designated as a, b, c, and d.

^b Examination of the comb while these bees were emerging showed them to be coming out of their cells neither sooner nor later on the average than their nonirradiated sisters.

WAVE LENGTH OF LIGHT INJURIOUS TO LARVAE

After it had been found that the light from both the carbon arc and the mercury-vapor lamp is very injurious to bee larvae, the next step was to determine what portion of this light contains the injurious rays. With the aid of the glass filters used in the investigation on adult bees it was possible to select that portion of the light having wave lengths longer than $313\text{ m}\mu$ (Pyrex filter), longer than $297\text{ m}\mu$ (Corex D filter), or between 265 and $405\text{ m}\mu$ (Corex A filter). These filters were used in irradiating larvae just hatched and larvae 1, 2, and 3 days old. All exposures were of 5 seconds' duration, and in all cases the comb was placed 31.5 inches from the lamp.

The results, given in table 10, show clearly that the light from the open lamp was the most harmful, that the light filtered through the Corex A glass was next most harmful, and that the light filtered through Corex D and Pyrex glass had a much less harmful effect. The light filtered through the Corex D glass was most harmful for larvae 1 and 2 days old, and that filtered through the Pyrex was the most harmful for the larvae just hatched or 3 days old.

The results of this experiment show that the harm done is not entirely a matter of total energy reaching the larvae; for although they received the most energy from the open lamp, they received the least from the light filtered through the Corex A filter, and the

light filtered through the latter did more harm than that filtered through either Corex D or Pyrex. However, to verify this, an experiment was performed in which the actual energy received by all the larvae was the same. Larvae of known age were obtained as already described. The comb was divided into areas at the time of irradiation by affixing an opaque screen over all except the area to be exposed. By using the rack illustrated in figure 3, the area of brood to be irradiated was placed at 72.6, 34.5, 35.9, or 15.7 inches from the lamp, depending on whether no filter at all, Pyrex, Corex D, or Corex A was used. All the larvae were 2 days old at the beginning of the experiment and were given 15 seconds' exposure then and 45 seconds' exposure on the following day. Counts of the individuals alive at the time of the last exposure and after 1, 2, 4, 7, 9, and 13 days showed whether or not the light had a lethal effect. The results are given in table 11.

TABLE 10.—The lethal effect on brood of different ages of 5 seconds' exposure to the total light from a mercury-vapor lamp, run at 4 amperes and 140 volts, and to three different spectral portions of it. Exposures made at 31.5 inches from the lamp

Age of larvae	Filter used	Number of larvae treated	Percentage of original number alive after indicated number of days		
			2	5	13
Just hatched...	None ..	21	100	15	9
	Pyrex	50	92	65	60
	Corex D ..	37	100	92	86
	Corex A ..	25	92	52	52
1 day	None	34	59	42	38
	Pyrex	34	100	94	94
	Corex D ..	20	90	75	75
	Corex A ..	18	94	39	39
2 days	None	24	0	0	0
	Pyrex	30	93	93	93
	Corex D ..	20	80	75	75
	Corex A ..	23	78	74	74
3 days	Pyrex	14	50	50	50
	Corex D ..	9	77	77	77

TABLE 11.—The lethal effect on honeybee larvae, 2 days old at the beginning of the experiment, of a total exposure of 1 minute and 15 seconds on the first day and 45 seconds 24 hours later, to the unfiltered light from a quartz mercury-vapor lamp, run at 4 amperes and 140 volts, and to three different spectral portions of this lamp, at such distances that the total energy reaching the larvae was the same in all exposures

Area irradiated	Filter used	Distance of comb from lamp	Larvae treated	Percentage of original number alive after indicated number of days					
				1	2	4	7	9	13 ^a
a	None	Inches	Number						
b	Untreated control		56	38	16	14	4	0	0
c	Pyrex	31.5	144	100	100	99	77	77	77
d	Corex D	34.5	91	99	98	98	98	98	97
e	Corex A	35.9	148	100	100	100	92	92	91
f	Corex A	15.7	179	35	2	2	1	1	1

^a Photograph of frame taken on the thirteenth day shown in fig. 5.

Both the unfiltered light and the light filtered through the Corex A glass killed practically all the larvae, while the light coming through the other glasses killed practically none of them, for no more died here than in the untreated area. This is shown strikingly in photographs taken of each side of the frame on the thirteenth day (fig. 5). Note that the brood exposed to the unfiltered light is all gone and

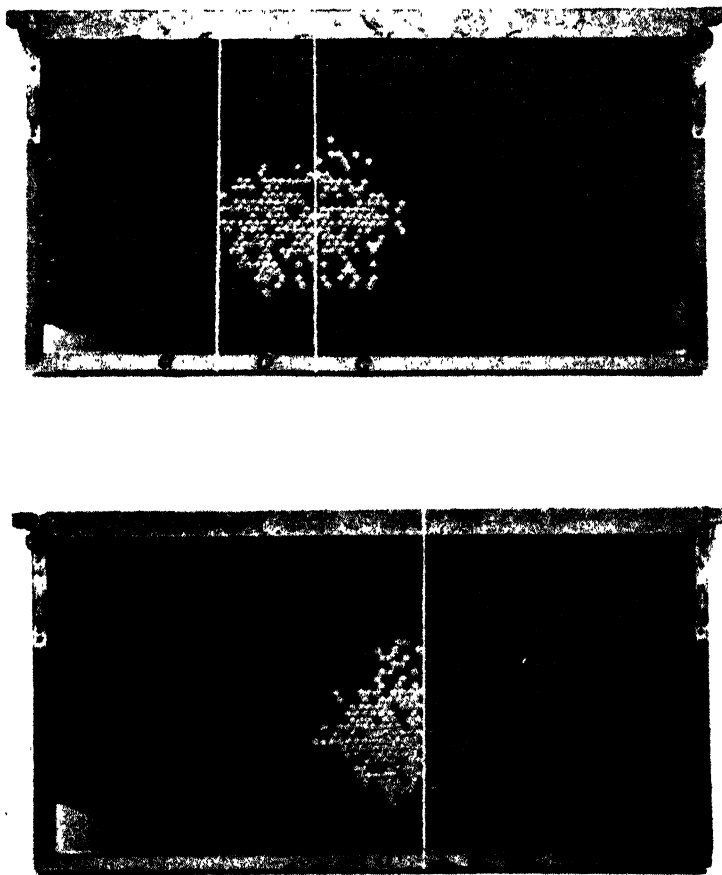


FIGURE 5.—Photographs of a frame of sealed brood in which different areas had been exposed to ultraviolet light when the larvae were 2 days old. The treatment of the various areas, lettered on the bottom bar of each frame, is given in table 11.

only one pupa remains in the area exposed to the light filtered through the Corex A glass. Since Corex A transmits rays down to 265 $m\mu$ wave length, whereas Corex D and Pyrex cut off the rays at 297 and 313 $m\mu$, respectively, it is evident that it is the shorter rays that kill the bees. In this experiment, also, the irradiated bees did not emerge any sooner than the others.

The general result of the experiments, then, is that ultra-violet radiations of wave length shorter than 297 $m\mu$ given in the dosages used here have a decidedly lethal effect on honeybee larvae and exhibit no beneficial effects at all.

EFFECT OF ULTRAVIOLET RAYS ON QUEEN LARVAE

Perhaps the main interest in this research centers in the effect of ultraviolet rays on queens, both larvae and adults. The observation of Muth¹² that a certain queen, which had been irradiated while in the larval stage, was extraordinarily large leads one to think that here may be found a subject capable of being benefited by these rays. Also, it should be easy to note the effect of these rays on the length of the developmental period and on the weight of the adult queen when she emerges.

Two experiments were performed to test the effect of ultraviolet rays on the length of the developmental period and on the weight of the adult queen when she emerges. Day-old larvae were transferred to queen cups and given to a queenless colony. Most of them were accepted by the colony at once and given liberal quantities of royal jelly. The next day the hive was opened, the cups were removed, and those containing living larvae were divided into four groups: Group 1 was kept unexposed to the ultraviolet rays as a control, group 2 was exposed to the unfiltered light from the quartz mercury-vapor lamp, group 3 to the same light filtered through the Corex D filter, and group 4 to the same light filtered through the Pyrex filter. As shown in table 1, groups 2, 3, and 4 were thus exposed to radiations extending into the ultraviolet as far as 185, 297, and 313 $m\mu$, respectively. In order to make the total energy received equal, these three groups were placed 72.6, 35.9, and 34.5 inches, respectively, from the lamp. In the first experiment the length of exposure on the first day was 5 seconds, and in the second experiment, 15 seconds. After the treatment the larvae were replaced in the hive. The next day they were given similar treatment except that the lengths of exposure were 15 and 45 seconds, respectively; they were then replaced in the hive as before. The following day the cells were sealed by the bees. Observations made from day to day showed whether or not the cells were still intact, an indication as to whether the baby queens inside were still alive. When it was nearly time for them to emerge, the queen cells were transferred to screen-wire cages such as are used by queen breeders, so that when a queen emerged she could not kill the queens within the other cells. By frequent examinations the time of emergence was ascertained fairly accurately. After emergence, in the second experiment, the queens were removed and weighed.

Table 12 shows that in both experiments the greatest mortality occurred when the larvae were irradiated with light from the unfiltered lamp; the next greatest mortality was among the larvae irradiated with the light filtered through the Corex D filter; while the least mortality was in experiment 1 among the controls and in experiment 2 among the larvae irradiated with light filtered through the Pyrex filter. Although the last-mentioned group seemed to receive some benefit from the light, the mortality among the controls was unusually high (33 percent) owing to unknown factors in the experiment, and hence this group probably received no actual benefit from the treatment.

¹² MUTH, C. F. See footnote 6.

* Numbers in parentheses refer to the number of adults having this developmental period

No great reliance should be placed upon the exact percentages of mortality in these groups because of the small number of larvae composing them, but the results do seem to accord with those given previously in this paper and indicate that the shorter ultraviolet rays have a decidedly harmful effect on queen larvae.

The light apparently had no effect at all on the length of the developmental period (table 12), as the average period for the controls was no longer or shorter than that for the others. Considerable variation was noted, due probably in part to the fact that there was a difference of perhaps as much as a day between the age of the youngest and of the oldest larva selected at the beginning of the experiment.

Although too few queens were weighed to warrant the drawing of important conclusions as to the effect of irradiation on the weight of the adult queen, the average weight of both lots of irradiated queens which survived the treatment was less than that of the nonirradiated controls.

Here again in the treatment of queen larvae no benefit resulted from irradiation with ultraviolet light.

EFFECT OF IRRADIATION ON ADULT QUEENS

For several years queen bees irradiated with ultraviolet light have been sold in the United States, and also of late in England. The claims usually made in favor of this treatment are that it makes the queens more prolific and that the resulting progeny are more gentle—that is, less inclined to sting—than bees from nonirradiated queens. But when one attempts to test these claims he is faced with many difficulties. In the first place the gentleness of a colony cannot be measured accurately, and even if it could, it varies greatly among different races and strains of bees and sometimes even among sister colonies, being dependent on such factors as the weather, the condition of the honey flow, and the skill of the beekeeper. The rate of egg laying can be measured with some degree of accuracy, however, and observations can be made as to the vitality of such irradiated queens and the frequency of supersedure among them. With these problems in mind, the following experiment was performed.

One hundred queens were purchased from a queen breeder in the South. These were shipped to Washington in the ordinary Benton mailing cages, and all arrived in apparently good condition. From Washington they were taken by automobile 50 miles north to Westminster, Md., where they were at once divided into five groups of 20 queens each. Group 1 was treated with unfiltered light from the quartz mercury-vapor lamp, the cages being placed 72.6 inches from the lamp tube; group 2 was treated with the same light filtered through the Pyrex filter, at a distance of 34.5 inches; group 3 was treated with the same light filtered through the Corex D (clear) filter, at a distance of 35.9 inches; group 4 was treated with the same light filtered through the Corex A (red-purple) filter 15.7 inches from the lamp; and group 5 was left untreated as a control. Each of the first four groups was further divided into 10 pairs, and each pair was given a different length of exposure, from one sixth minute on each of the first three alternate days up to 10 minutes on the first and third days. Exposures were

made by placing the cages, wire-screen side toward the lamp, at the specified distance from the lamp tube for the specified length of time. Considerable heat was given out by the lamp, but the temperature at the cages, even after 10 minutes' exposure at 15.7 inches (using the Corex A filter), the shortest distance used, never rose above 30° C. (86° F.), which was below the temperature within the hive. Each day after the exposures were made the queen cages were hung in a special frame and placed in a hive of bees so that they might be kept at normal temperature and perhaps be given extra food by the workers. The cages containing the controls were hung in the same special frame and were thus taken from the hive every day along with those to be treated, and in all other respects were handled as nearly as possible like the cages containing the treated queens except that they were not exposed to the light.

Meanwhile, 100 2-pound packages of bees had been ordered and, through the cooperation of a commercial beekeeper, 100 hives had been prepared, each consisting of a single story with nine frames of empty drawn comb.

The treatments were administered between April 15 and 20, 1930. On April 23 the 2-pound packages arrived and were released into the hives, and with each of them was placed a queen cage containing a queen and several workers. In such a method of introducing queens the hole at one end of the cage is plugged with soft candy, which in the course of 2 to 3 days is eaten out by the workers in the hive, and the queen is thereby released.

Examination of the hives was then begun and continued for 3½ months, at first at intervals of approximately 1 week, but toward the last, of 2 weeks. Notice was taken particularly as to whether the original queen was still present in the hive or was being superseded by another. This could easily be ascertained, because before supersedure the bees build a series of queen cells and start rearing queens therein. Then, either during the development of these new queens in their queen cells or shortly before, the workers kill the old queen. Consequently, upon examining a hive in the process of supersedure, one usually finds several queen cells and at the same time notes a lack of eggs and very young larvae and the absence of the old queen.

Three times while the colonies were under observation measurements were made of the area of brood in the combs. The first measurement, after 43 days, was of sealed brood only, but in the other two, after 70 and 108 days, respectively, the entire brood was measured. This was done with a ruler; since the brood ordinarily occupies an elliptical area on each side of a comb, by measuring the length and breadth of this ellipse the area of the brood it contained could easily be computed.

The detailed results of this experiment are given in table 13, which shows the treatment given each of the 100 queens, whether or not she was alive at each examination both before and after introduction into the colonies, and, if she survived, gives the area of comb occupied by her brood at the time the different measurements were made.

GROUP 4. COREX A FILTER, 15.7 INCHES FROM LAMP

[illegible]

GROUP 5 CONTROLS, UNTREATED

[illegible]

• All control queens were alive 6 days after the start of the experiment.

SURVIVAL OF QUEENS

A large number of queens died during the treatment and before introduction into the colonies, only 42 surviving beyond this period. Such a high mortality need not always occur in an experiment of this kind, however. A number of unfavorable circumstances combined here to place the queens in a weakened physical condition—for example, a delay in delivery by the post, a ride of 50 miles by automobile on a chilly day, and inferior queen-cage candy that became too dry and hard toward the last, a condition which was not noticed in time to remedy it. Moreover, a subsequent test showed that under more favorable conditions queens may be kept in Benton cages for at least 2 weeks and during that time be given a total of as much as 1½ hours of irradiation and still survive apparently in good condition. But since in the present experiment the queens were treated as nearly alike as possible except for the irradiation and although weakened at the start were equally weakened, the results for the various groups are considered comparable.

When the number of survivals in the various groups (table 13) are compared, we find that the queens of group 2 survived far better than did those of any other group. These were the queens treated with light filtered through the Pyrex filter, and since Pyrex transmits only wave lengths of 313 $m\mu$ or longer, one may conclude that the screening out of the shorter rays saved many of the queens from death. As a matter of fact, a few more queens of group 2 survived than of the untreated controls (group 5), which indicates that the light filtered through Pyrex had either a beneficial effect or at least no harmful effect.

As a rule more queens died that received the longer treatments than those that received the shorter. For instance, not 1 of the 4 introduced queens that were exposed 20 minutes survived more than 1 week after introduction into the colony. As a matter of fact, if a comparison is made between all the queens receiving irradiation of 3 minutes or less and those exposed for 6 to 20 minutes, it will be found that 50 percent of the former survived until introduction and 62 percent of those introduced survived until the end of the experiment; whereas, of the latter, only 33 percent survived until introduction and only 39 percent of these survived until the end of the experiment.

A further result of this experiment, shown in table 13, is that up to the time of introduction about the same percentage of irradiated queens died as of nonirradiated controls, but that after introduction the supersedure was considerably greater among the irradiated queens. A count will show that of 30 irradiated queens introduced successfully (as evidenced by the fact that they were alive 1 week after introduction) to colonies, 12, or 40 percent, were superseded, while of the 8 nonirradiated queens introduced only 2, or 25 percent, were superseded. Some of the treated queens (nos. 25a, 25b, 26a, and 29b) died even before introduction, on the second day after the start of the experiment, whereas all the controls lived 6 days, indicating that the treatment had some harmful effect.

The net result of the experiment, therefore, is that irradiated queens have practically as good a chance of survival as nonirradiated ones kept during the same period, but that after introduction to colonies nonirradiated queens have a somewhat better chance of survival.

AREA OF BROOD

As to the area of brood produced by each queen, table 14 shows a slight advantage for the irradiated queens. For the 12 surviving queens which had received 3 minutes' or less irradiation (13 survived until the end of the experiment, but 1 of these produced only drone brood and was not included in these measurements), the average area of brood for the three examinations was 494 square inches and for the 5 which had received 6 to 20 minutes' irradiation, 474 square inches, making an average of 488 square inches for the 17 treated queens as compared with 449 square inches for the 6 untreated controls. From these data, if we disregard the errors inherent in this method of measuring the brood, it appears that the treated queens produced about 9 percent more brood than did the untreated queens. The same tendency was noted each time the area of brood was measured. On the forty-third day after introduction the advantage held by the irradiated queens was 9 percent, on the seventieth day about 7 percent, and on the one hundred and eighth day about 12 percent. Of the four groups of irradiated queens those of group 1 produced the most brood, although the number of queens surviving in most of the groups is too small to merit attaching much importance to the comparison.

As far as one may conclude from this experiment, then, the practice of irradiating queens with ultraviolet light is of no practical value to the beekeeper, for although it may result in a slightly greater rate of egg laying, there is also somewhat more supersedure among the queens during their first few months.

TABLE 14.—*Summary of data given in table 13, showing the average area of brood^a produced by the queens receiving 3 minutes or less of treatment and those receiving from 6 to 20 minutes of treatment, together with the sum of the two groups as compared with the brood produced by queens receiving no treatment at all*

Group	Irradiated 3 minutes or less		Irradiated 6 to 20 minutes		Total for all irradiated queens		Nonirradiated queens	
	Number	Average area	Number	Average area	Number	Average area	Number	Average area
	3	<i>Sq in.</i> 544	1	<i>Sq in.</i> 492	4	<i>Sq in.</i> 531		<i>Sq in.</i>
	6	497	2	430	8	480		
	1	345	1	480	2	413		
	2	483	1	537	3	501		
Total or average	12	494	5	474	17	488	6	449

^a The data given are averages of the measurements made on the forty-third, seventieth, and one hundred and eighth days after introduction.

SUMMARY AND CONCLUSIONS

The effect of irradiation with ultraviolet light on larvae and adults of both worker and queen honeybees was tested. Adult workers were irradiated in cages by exposing them to light from a quartz mercury-vapor lamp. The distance from the lamp, the length of exposure, and the wave length of the light were varied (the last by means of filters) in a great many combinations in the different tests. The effect of the irradiation was measured by ascertaining the average length of

life of the bees in each cage. The results showed that ultraviolet light, given in the larger dosages, is definitely harmful to bees. When the light was given in smaller quantities there was, in a few experiments, an apparently beneficial effect, but the results here were not consistent with those in other experiments; hence, the conclusion is that neither the total light from the lamp (185 to 1,400+ $m\mu$ wave length) nor that portion of the light above 313 $m\mu$, nor that portion above 297 $m\mu$, nor that portion between 265 and 405 $m\mu$ has, in the dosages here used, any significant beneficial effect on the longevity of bees. Irradiation of the food (queen-cage candy) did not lengthen the average span of life.

Irradiation of worker larvae showed that they are easily harmed by ultraviolet rays, especially at the wave lengths shorter than 297 $m\mu$, brood 3½ days old or younger being more susceptible than brood 4½ days of age or older. No beneficial effects were observed.

Similar results were obtained when queen larvae were irradiated. The effect of the shorter rays (below 297 $m\mu$) proved decidedly harmful, while the longer rays had little or no effect. There was no shortening of the developmental period by any sort of irradiation used, and no significant difference in the weight of the emerging queens.

The irradiation of adult queens seemed to reduce their vitality somewhat, especially after the longer exposures, 6 to 20 minutes. The rate of egg laying of the irradiated queens was slightly greater, but any practical value to be derived from this is offset by the fact that there was considerably more supersedure among such queens.

The general harmful effect of the shorter ultraviolet rays, noted rather consistently in these experiments, together with the absence of any general good effect (except the slight increase in rate of egg laying by queens), makes it appear doubtful if irradiation of honey-bee larvae or adults by ultraviolet light is commercially valuable.

EFFECT OF STAND IRREGULARITIES UPON THE ACRE YIELD AND PLANT VARIABILITY OF CORN¹

By T. A. KIESSELBACH, *agronomist*, and RALPH M. WEIHING, *experimental assistant, Nebraska Agricultural Experiment Station*

INTRODUCTION

The importance of extreme uniformity in the spacing of crop plants growing under field conditions is still a debatable question. An investigation of this factor in the production of corn (*Zea mays* L.) has been concluded at the Nebraska experiment station, and the results of 14 years' work are summarized in this paper. In a recent publication bearing upon this question Sprague and Farris² have cited a bibliography of 13 papers.

The extent to which variability in the development of individual corn plants is induced by stand irregularities is a phase of the problem not previously investigated. The procedure followed in the variability study was to measure large populations, grown under specified variable stand conditions, with respect to different characters, including (1) plant height at several stages of development, (2) ear height, (3) stalk diameter at first internode aboveground, (4) number of days from planting to silking, and (5) grain yield per ear-bearing plant. The standard deviations and coefficients of variation were calculated from these individual plant data.

Under farm conditions, stand irregularities associated with planting practices may result from such causes as imperfect germination, mechanical imperfections of the corn planter, and nonuniformity of seed size. According to tests conducted at the Nebraska station,³ equally as good results may be expected whether the plants are individually spaced in rows, as in a drilled field, or whether they are grown in hills, as in a checked field. The stand which the corn grower strives to obtain is designed to occupy the land fully and to utilize all the field resources for crop growth. The usual aim is to have the plants equidistant in drills, or to have a constant number of plants in hills spaced at regular intervals.

KINDS OF STANDS COMPARED

In these studies the aim was to have an equal number of plants per acre, distributed both regularly and irregularly in hills 42 inches apart each way. The only variable factor in the different plantings under comparison was in the degree of irregularity of the stand. The variation in plant distribution was brought about by two methods, known herein as systematically variable and randomly variable planting. (1) In the experiment with systematically variable distri-

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² SPRAGUE, H. B., and FARRIS, N. F. THE EFFECT OF UNIFORMITY OF SPACING SEED ON THE DEVELOPMENT AND YIELD OF BARLEY. Jour. Amer. Soc. Agron. 23:516-533, illus. 1931.

³ KIESSELBACH, T. A., ANDERSON, A., and LYNESS, W. E. TILLAGE PRACTICES IN RELATION TO CORN PRODUCTION. Nebr. Agr. Expt. Sta. Bul. 232.9. 1928.

bution, which was continued for 14 years, successive hills in the row were made to differ systematically in number of plants by planting sound seed of a standard local variety of dent corn at a double rate and thinning to the desired number. The following stands were compared: (a) Uniformly 3 plants per hill, (b) alternating 2- and 4-plant hills, (c) alternating 1-, 3-, and 5-plant hills, and (d) alternating 1-, 2-, 3-, 4-, and 5-plant hills. (2) In 1931 an additional comparison was made of randomly variable distributions in which the various stands were obtained by the use of four lots of seed having the respective germination percentages of 100, 75, 60, and 50. By planting these various samples at the rate of 3, 4, 5, and 6 seeds per hill, respectively, the number of plants per acre was approximately 10,668 in each instance, but the number of plants per hill was variable.

To obtain the specific germination desired, the seed samples were prepared by thoroughly mixing sound and dead seed in the necessary proportions. The dead seeds were identical with the sound seeds, except that their viability had been destroyed by heat for the purpose of this test.

Typical illustrations of stands obtained in both the systematically and randomly variable distributions are presented in figures 1 and 2. It does not appear that imperfections in any modern corn-planting device or nonuniformity in seed size could result in greater stand irregularities than have occurred here.

COMPARATIVE VARIABILITY OF STANDS

In the study of systematically variable distributions the plantings consisted of duplicate 4-row plots, 16 rods long, except in 1931 when there were six replications of 5-row plots 12 hills long. The plots of the randomly variable distributions were replicated 15 times and consisted of 4 rows 12 hills long. Border rows were discarded and the results were based on the interior rows.

In the case of the systematically variable stands it was especially difficult to visualize comparative variability; that is, to determine just how these stands rank as to nonuniformity, and how they compare with those obtained from adjusted planting rates with seed of various germination percentages. In order to appraise these differences the average deviations were calculated for all of the plant distributions, as shown in tables 1 and 2. From the data there presented it is evident that of the systematic distributions, the alternating 1-, 3-, and 5-plant hills give the least uniform stand and that an alternation of 1-, 2-, 3-, 4-, and 5-plant hills gives a more variable stand than is secured from an adjusted planting rate with seed having a germination as low as 50 percent.

TABLE 1.—Average deviations from the mean number of plants per hill in the systematically variable plant distributions, 1915-17, 1920-29, and 1931

Type of stand	Distribution of plants in successive hills	Mean	Average deviation
		Number	Number
Uniform.....	3-3-3-3.....	3	0.00
Uneven.....	2-4-2-4.....	3	1.00
Do.....	1-2-3-4-5.....	3	1.20
Do.....	1-3-5.....	3	1.33

UNIFORMLY 3-PLANT HILLS											
3	3	3	3	3	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	3

ALTERNATING 2-AND 4-PLANT HILLS											
2	4	2	4	2	4	2	4	2	4	2	4
4	2	4	2	4	2	4	2	4	2	4	2
2	4	2	4	2	4	2	4	2	4	2	4
4	2	4	2	4	2	4	2	4	2	4	2
2	4	2	4	2	4	2	4	2	4	2	4

ALTERNATING 1-3-AND 5-PLANT HILLS											
1	3	5	1	3	5	1	3	5	1	3	5
3	5	1	3	5	1	3	5	1	3	5	1
5	1	3	5	1	3	5	1	3	5	1	3
1	3	5	1	3	5	1	3	5	1	3	5
3	5	1	3	5	1	3	5	1	3	5	1

ALTERNATING 1-2-3-4-AND 5-PLANT HILLS											
1	2	3	4	5	1	2	3	4	5	1	2
2	3	4	5	1	2	3	4	5	1	2	3
3	4	5	1	2	3	4	5	1	2	3	4
4	5	1	2	3	4	5	1	2	3	4	5
5	1	2	3	4	5	1	2	3	4	5	1

FIGURE 1.—Diagram showing systematic distribution of hill types within 5-row plots. Numbers indicate the number of plants per hill.

PLOT NO 4782 SEED GERMINATING 75 PERCENT DROPPED AT 4 KERNELS PER HILL												
ROW 1	3	3	3	3	4	2	3	3	4	2	4	4
ROW 2	3	3	2	4	4	1	2	4	3	3	2	2
ROW 3	4	4	3	3	4	2	3	2	3	3	4	3
ROW 4	3	3	3	3	4	2	3	1	4	3	2	4

PLOT NO 4783 SEED GERMINATING 60 PERCENT DROPPED AT 5 KERNELS PER HILL												
ROW 1	3	3	4	4	2	2	3	5	5	4	4	4
ROW 2	2	3	3	1	3	4	2	4	4	2	4	2
ROW 3	4	3	3	4	3	2	2	3	3	2	2	3
ROW 4	4	5	2	1	4	3	3	5	4	4	2	4

PLOT NO 4784 SEED GERMINATING 50 PERCENT DROPPED AT 6 KERNELS PER HILL												
ROW 1	3	1	2	3	5	4	5	3	3	2	2	1
ROW 2	2	5	6	4	4	3	5	4	2	4	3	2
ROW 3	2	4	3	4	3	3	2	5	3	1	3	5
ROW 4	1	2	2	3	2	2	5	4	4	2	5	0

PLOT NO. 4785 SEED GERMINATING 100 PERCENT DROPPED AT 3 KERNELS PER HILL												
ROW 1	3	3	3	3	3	3	3	3	3	3	3	3
ROW 2	3	3	3	3	3	3	3	3	3	3	3	3
ROW 3	3	3	3	3	3	3	3	3	3	3	3	3
ROW 4	3	3	3	3	3	3	3	3	3	3	3	3

FIGURE 2.—Diagram of four representative field plots, showing the number of plants in successive hills obtained from four lots of seed differing in germination percentage and planting rate. Numbers indicate the number of plants in each hill.

TABLE 2.—Average deviations from the mean number of plants per hill in the randomly variable plant distributions, 1931

Percentage germination of seed planted		Seeds dropped per hill	Mean	Average deviation
Laboratory	Field			
		Number	Number	Number
100	96.85	3	2.90	0.18
75	72.67	4	2.90	.73
60	58.50	5	2.91	.85
50	49.68	6	2.98	1.00

ACTUAL v. THEORETICAL STANDS FROM IMPERFECTLY GERMINATING SEED

Special interest attaches to the randomly variable stands in 1931 with respect to their approach in actual number of various hill types to the theoretically expected number. After proper technic had been employed to procure random distributions, seedling counts were made and recorded for each individual hill. Since the actual field germination and the number of seeds planted per hill were now known, it was possible by applying the law of chance to compute the frequency with which different hill types might be expected to occur in the various random distributions.

Under the conditions of this experiment four plant distributions were obtained from seed germinating 100, 75, 60, and 50 percent, planted 3, 4, 5, and 6 seeds per hill, respectively (table 2). The actual field germination based on seedling counts was 96.85, 72.67, 58.50, and 49.68 percent for the respective lots of seed. With these data, it was possible to determine the expected number of 0-, 1-, 2-, 3-, 4-, 5-, and 6-plant hills to be compared with the observed number. These data are presented in table 3. The formula employed in this comparison, and previously used by Kiesselbach and Ratcliff,⁴ follows. The probability of dropping p sound kernels and q dead kernels from a composite sample containing m sound kernels and n dead kernels is:

$$\frac{m'}{(m-p)'} \frac{(m+n-p-q)'}{(m+n)'} \frac{n'}{(n-q)'} \frac{(p+q)'}{p' q'}$$

where $m' = m(m-1)(m-2)(m-3) \dots 3 \cdot 2 \cdot 1 =$ product of all integers from 1 to m .

If the χ^2 test for goodness of fit is applied to these data, the values of P are 0.4, 0.1, 0.8, and 0.2 for seed germinating nearly 100, 75, 60, and 50 percent, respectively. These values show that the deviations of the observed from the expected were not excessively large, and that imperfect germination and rate of planting account for the frequency distribution of the various hill types. In computing the foregoing values of P , the hill types were so grouped that no expected class contained fewer than five hills in accordance with the procedure suggested by Fisher.⁵

⁴ KIESELBACH, T. A., and RATCLIFF, J. A. FREEZING INJURY OF SEED CORN. Nebr. Agr. Expt. Sta. Research Bul. 16: 92. 1920.

⁵ FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 p., illus. Edinburgh and London. 1930.

TABLE 3.—Frequency of various hill types resulting from seed germinating 100, 75, 60, and 50 percent,* planted at specified number of seeds per hill, 1931

FIELD GERMINATION 96.85 PERCENT AND 3 KERNELS DROPPED PER HILL						
Plants per hill	Number of hills			Percentage of hills		
	Observed	Expected	Deviation	Observed	Expected	Deviation
0	1	0.02	0.98	0.139	0.003	0.136
1	8	2.06	5.95	1.113	.285	.828
2	50	63.83	-13.83	6.964	8.878	-1.924
3	660	653.10	6.90	91.794	90.834	.960
FIELD GERMINATION 72.67 PERCENT AND 4 KERNELS DROPPED PER HILL						
0	8	3.99	4.01	1.113	0.555	0.558
1	51	42.59	8.41	7.093	5.923	1.170
2	157	170.22	-13.22	21.836	23.674	-1.838
3	288	301.83	-13.83	40.055	41.980	-1.925
4	215	200.37	14.63	29.903	27.868	2.035
FIELD GERMINATION 58.50 PERCENT AND 5 KERNELS DROPPED PER HILL						
0	7	8.76	-1.76	0.979	1.225	-0.246
1	65	61.93	3.07	9.091	8.661	.430
2	174	174.90	-.90	24.336	24.462	-.126
3	257	246.71	10.29	35.944	34.505	1.439
4	161	173.79	-12.79	22.517	24.306	-1.789
5	51	48.91	2.09	7.133	6.841	.292
FIELD GERMINATION 49.68 PERCENT AND 6 KERNELS DROPPED PER HILL						
0	18	11.64	6.36	2.503	1.619	0.884
1	70	69.10	.90	9.736	9.611	.125
2	172	170.73	1.27	23.922	23.746	.176
3	207	224.81	-17.81	28.790	31.267	-2.477
4	102	166.36	-64.36	22.531	23.137	-.606
5	79	65.60	13.40	10.988	9.123	1.865
6	11	10.76	.24	1.530	1.497	.033

* Field germination was slightly less than the designated percentages.

EFFECT OF UNEVEN STANDS UPON THE ACRE-YIELD OF GRAIN

SYSTEMATICALLY VARIABLE STANDS

The annual and average yields secured during 14 years from three systematically variable plant distributions where the number of plants per acre has remained constant and the number of plants per hill has varied systematically, are reported in table 4 in comparison with a uniform stand. The average of the three uneven distributions was 50 bushels as compared with 49.9 bushels for uniformly 3-plant hills. Under the conditions of these extreme variations in stand the actual yield has not been significantly affected.

A summary of yield and various other agronomic characters is given in table 5. It is evident that these inequalities of stand have not, to any important degree, affected the general development of the crop.

TABLE 4.—*Effect of systematically variable plant distribution upon the yield in bushels per acre of corn, 1915-17, 1920-29, and 1931**

Year	Yield per acre from plants in successive hills (10,668 plants per acre) in—				
	Variable stands				Uniformly 3-plant stands
	Alternat- ing 2 and 4 plants	Alternat- ing 1, 3, and 5 plants	Alternat- ing 1, 2, 3, 4, and 5 plants	Average for uneven stands	
1915	89.8	88.0	95.1	91.0	93.4
1916	58.4	52.4	54.4	55.1	55.2
1917	28.1	26.3	30.6	28.3	26.7
1920	51.9	49.9	50.2	50.3	52.3
1921	68.0	64.3	62.9	65.1	66.7
1922	45.1	45.9	45.4	45.5	44.7
1923	69.8	65.0	65.4	66.7	70.9
1924	43.9	43.2	44.1	43.7	40.9
1925	42.0	43.0	41.9	42.6	39.2
1926	24.7	23.4	23.2	23.8	22.4
1927	52.2	53.1	53.0	53.1	53.1
1928	47.6	50.8	46.1	48.2	44.1
1929	60.3	60.9	61.1	60.8	64.2
1931	26.3	24.3	25.8	25.5	24.2
Average	50.6	49.3	50.0	50.0	49.9

* Tests made in duplicate 4-row plots 16 rods long, except in 1931, when there were 6 replications of 5-row plots, 12 hills long. Yields are calculated on the basis of shelled corn containing 14 percent moisture

TABLE 5.—*Effect of systematically variable distribution of corn plants upon various agronomic characters, during 14 years,* 1915-17, 1920-29, and 1931*

[In tassel, Aug. 3; ripe, Sept. 22]

Type of stand	Distribu- tion of plants in successive hills	Height		Per 100 plants					Shrink- age	Shell- ing	Yield per acre
		Stalk	Ear	Smut- ted	Barren	2-eared	Suck- ers	Lodged			
		Inches	Inches	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Percent	Percent	Bush- els
Uneven	2-4-2-4	93.7	45.9	7.9	6.8	8.6	16.4	16.7	7.4	82.7	50.6
Do.	1-3-5	94.4	44.5	7.8	7.9	8.9	18.3	14.8	6.7	82.9	49.3
Do.	1-2-3-4-5	93.8	44.7	8.8	8.6	9.7	21.0	13.7	6.9	83.4	50.0
Average		94.0	45.0	8.2	7.8	9.1	18.6	15.1	7.0	83.0	50.0
Uniform	3-3-3-3	94.6	45.5	8.5	6.8	8.4	15.1	15.8	7.4	82.8	49.9

* Data on vegetative characters are averages for 10 years only.

RANDOMLY VARIABLE STANDS

For the crop grown in 1931 from lots of seed germinating 100, 75, 60, and 50 percent planted at the rate of 3, 4, 5, and 6 seeds per hill, respectively, the corresponding yields (table 6) were 24.96 ± 0.65 , 25.50 ± 0.66 , 25.34 ± 0.66 , and 25.12 ± 0.65 bushels per acre. Any differences in yield are within the probable error, and it is concluded that these variable stands have not affected acre yields. Other agronomic characteristics of the crop have been so little changed as to be of no very material consequence.

TABLE 6.—*Effect of randomly variable distribution of corn plants upon various agronomic characters and yield, 1931*^a

Percentage germination of seed planted	Seeds dropped per hill	Per 100 plants				Yield per acre
		Barren	2-eared stalks	Suckers	Broken or lodged	
	Number	Number	Number	Number	Number	Bushels
75	4	26 6	3 1	6 3	0 9	25.50±0.66
80	5	22 4	3 4	5 6	. 8	25.34±.66
50	6	27 1	2 5	4 4	9	25.12±.65
Average		25 4	3 0	5 4	9	25.32±.38
100	3	22 4	2 4	4 5	. 7	24.96±.65

^a Averages for 15 replications of 4-row plots 12 hills long. Yields are calculated on the basis of shelled corn containing 14 percent moisture

EFFECT OF UNEVENNESS OF STAND UPON THE DEVELOPMENT AND VARIABILITY OF CORN PLANTS

CALCULATION OF BIOMETRICAL CONSTANTS

The influence of stand irregularities upon different plant characters as measured by the standard deviation and coefficient of variation was determined in 1931. These constants are reported in the succeeding variability tables, together with the mean value for the character concerned.

Differences between the constants for the variable and the uniform stands are reported in the lower part of each table. These differences will suggest how much more or less variable are the uneven than the even stands. Statistical significance is expressed in the tables only by the D/PE (the difference divided by the probable error of the difference) calculations. In the analysis of these data statistical significance is attached to any difference which is approximately three times its probable error. When the chances of exceeding a given difference in one direction only are considered, this is equivalent to odds of about 39:1, or if the chances of exceeding a given difference in either direction are considered, the odds are 19:1.

The formulas used in calculating the various constants are as follows:

(1) Standard deviation =

$$\sqrt{\frac{\sum fd^2}{n} - \frac{S(fd)^2}{n^2} - c/12}$$

where n = number of variates, fd = frequency $\times$ deviation from arbitrary origin, and $c/12$ = Sheppard's correction for grouping according to Ezekiel.⁶

(2) Combined standard deviation =

$$\sqrt{\frac{S_1(x_1 - \bar{x}_1)^2 + S_2(x_2 - \bar{x}_2)^2}{(N_1 - 1) + (N_2 - 1)}}$$

where $S_1(x_1 - \bar{x}_1)^2$ = sum of squares of the deviations from the sample mean and N_1 = number of variates.⁷

All other statistical constants employed were computed in accordance with standard, authoritative procedure.

⁶ EZEKIEL, M. METHODS OF CORRELATION ANALYSIS. 427 p., illus. New York and London. 1930.

⁷ TIPPETT, L. H. C. THE METHODS OF STATISTICS; AN INTRODUCTION MAINLY FOR WORKERS IN THE BIOLOGICAL SCIENCES. 222 p., illus. London. 1931.

MATURE PLANT HEIGHT

At maturity (tables 7 and 8) plant-height measurements were taken in inches from the ground level to the lowest tassel branch. In all cases, with both types of variable distributions, mean plant height has been depressed a fraction of an inch. The average reduction was 0.81 inch in the case of the systematic and 0.56 inch in the case of the randomly variable distributions. These differences have D/PE values of 3.38 and 3.11, respectively, and are suggestive of reduction in mean plant height. The differences are so small, however, as to have no agronomic importance.

TABLE 7.—*Effect of systematically variable plant distribution upon the plant height of corn and its variability at maturity, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Type of stand	Distribution of plants in successive hills	Plants measured	Height of plants		
			Mean	Standard deviation	Coefficient of variation
		Number	Inches	Inches	
Uneven.....	2-4-2-4.....	627	69 50	8 01	11.52
Do.....	1-3-5.....	624	69 70	8 27	11 86
Do.....	1-2-3 4-5.....	638	69 47	8 54	12 29
Average *.....			69 56	8 20	11 92
Uniform.....	3-3-3-3.....	633	70 37	7 29	10 36

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE UNIFORM STAND

Distribution of plants in successive hills	Height of plants					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Inches		Inches		Inches	
2-4-2-4.....	-0 87	2 90	0 72	3 60	1 16	3 87
1-3-5.....	-.67	2 23	.98	4 67	1 50	5 00
1-2-3-4-5.....	- 90	3 00	1 25	5 95	1 93	6 22
Average ^b	- 81	3 38	1 00	5 88	1 56	6 50

* For the averages, a weighted mean and combined standard deviation have been calculated and the coefficient of variation was derived from them.

^b The average differences were found by comparing the weighted mean, combined standard deviation, and coefficient of variation with the corresponding constants of the normal distribution

TABLE 8.—*Effect of randomly variable plant distribution upon the plant height of corn and its variability at maturity, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Percentage germination of seed planted	Seeds dropped per hill	Plants measured	Height of plants		
			Mean	Standard deviation	Coefficient of variation
	Number	Number	Inches	Inches	
75.....	4	1,013	68.75	8.12	12.16
60.....	5	960	67.52	7.88	11.67
50.....	6	1,052	68.76	8.06	12.07
Average *.....			67.00	8.03	11.98
100.....	3	993	67.56	7.10	10.51

TABLE 8.—*Effect of randomly variable plant distribution upon the plant height of corn and its variability at maturity, 1931—Continued*

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE NORMAL STAND

Percentage germination of seed planted	Height of plants					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Inches		Inches		Inches	
75.....	-.81	3.52	1.02	6.38	1.65	6.88
60.....	-.04	17	.78	4.88	1.16	4.83
50.....	-.80	3.48	.96	6.00	1.56	6.50
Average *.....	-.56	3.11	.93	7.15	1.47	7.74

* Refer to table 7, footnotes.

Variability in plant height, as indicated by the standard deviation and coefficient of variation, was slightly greater in the variable distributions. The combined standard deviations for the variable stands of both types of planting were significantly larger than the standard deviations for the uniform stands. The increases were 1 inch for the systematic and 0.93 inch for the random distributions. The coefficients of variation corroborate the standard deviations in this respect.

While mature plant height was slightly reduced and variability increased because of a lack of uniformity in stands, the differences were too small to have much agronomic importance.

PLANT HEIGHT AT IMMATURE STAGES

Plant-height measurements were taken on all the plants in the systematically variable distributions 7 and 9 weeks after planting. Similar records were made for the random distributions 4 and 7 weeks after planting. Measurements were taken to the maximum height when the leaves were held erect and are designated as "stretched height". For the sake of brevity, since no material differences were found, the detailed results are not given in table form.

Seven weeks after planting, when the stretched height was approximately 48 inches, the mean height of the plants grown in systematically variable distributions was 0.18 ± 0.18 inch shorter; the combined standard deviation 0.12 ± 0.12 inch less, and the coefficient of variation 0.21 ± 0.26 percent less than for plants grown in uniformly 3-plant hills. Nine weeks after planting, when the stretched height of plants was about 72 inches, the mean height was 0.56 ± 0.22 inch shorter; the combined standard deviation 0.43 ± 0.15 inch larger, and the coefficient of variation 0.67 ± 0.22 percent larger as an average for all three systematically variable distributions than for plants grown in uniformly 3-plant hills. These differences approach significance, but are too slight to have importance.

For plants resulting from seed germinating 100, 75, 60, and 50 percent planted at compensating rates, the mean height (stretched) of approximately 36 inches 4 weeks after planting was 0.27 ± 0.09 inch less; the combined standard deviation 0.58 ± 0.07 inch larger, and the coefficient of variation 1.72 ± 0.19 percent larger for plants

grown from imperfectly germinating seed than for those resulting from seed germinating 100 percent. At 7 weeks after planting, when stretched height was about 61 inches, the mean height was 0.81 ± 0.14 inch shorter; the combined standard deviation 0.39 ± 0.09 inch larger, and the coefficient of variation 0.77 ± 0.16 percent larger for plants resulting from imperfectly germinating seed than for those resulting from seed germinating 100 percent. These differences are statistically significant but not important agronomically.

EAR HEIGHT

The height of ears was measured from the ground level to the ear node of the stalk. There was an average increase of 0.18 inch for mean ear height in case of the systematic and a decrease of 0.10 inch in case of the randomly variable stands. These differences (tables 9 and 10) have D/PE values of 1 and 0.67, respectively, and are not statistically significant.

The combined standard deviations and coefficients of variation for the variable stands were not significantly different from those for the uniform stands. Thus no important ear-height effects resulted from these stand irregularities.

TABLE 9.—*Effect of systematically variable plant distribution upon the ear height of corn and its variability, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Type of stand	Distribution of plants in successive hills	Plants measured	Ear height		
			Mean	Standard deviation	Coefficient of variation
			Inches	Inches	
Uneven.....	2-4-2-4.....	610	37.08	5.78	15.59
Do.....	1-3-5.....	599	37.95	5.84	15.39
Do.....	1-2-3-4-5.....	621	37.35	6.26	16.76
Average *.....			37.46	5.97	15.94
Uniform.....	3-3-3-3.....	615	37.28	5.79	15.53

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE UNIFORM STAND

Distribution of plants in successive hills	Ear height					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
2-4-2-4.....	Inches -0.20	0.87	Inches -0.01	0.06	0.06	0.14
1-3-5.....	.67	2.91	.05	.31	-.14	.32
1-2-3-4-5.....	.07	.30	.47	2.94	1.23	2.80
Average *.....	.18	1.00	.18	1.38	.41	1.17

* Refer to table 7, footnotes.

TABLE 10.—*Effect of randomly variable plant distribution upon the ear height of corn and its variability at maturity, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Percentage germination of seed planted	Seeds dropped per hill	Plants measured	Ear height		
			Mean	Standard deviation	Coefficient of variation
			Inches	Inches	
75.....	4	949	40.29	5.75	14.27
60.....	5	912	40.17	6.10	15.18
50.....	6	919	40.05	5.54	13.83
Average ^a			40.17	5.80	14.44
100.....	3	934	40.27	5.72	14.20

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE NORMAL STAND

Percentage germination of seed planted	Ear height					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Inches		Inches			
75.....	0.02	0.11	0.03	0.23	0.07	0.22
60.....	— .10	.53	.38	2.92	.98	2.97
50.....	— .22	1.22	.18	1.38	— .37	1.16
Average ^a	— .10	.67	.08	.80	.24	.92

^a Refer to table 7, footnotes.

STALK DIAMETER

Stalk-diameter determinations were made only upon the plants in the randomly variable distributions, or the plants resulting from seed corn with imperfect germination. This character, apparently, was definitely affected by uneven plant distribution. The results are presented in table 11.

The mean stalk diameter for plants grown from seed germinating 100 percent was 23.72 mm, while for plants in the variable distributions, grown from imperfectly germinating seed, the average mean diameter was 23.14 mm. These values show a decrease of 0.58 mm in stalk diameter for plants of the irregular stands. In every instance mean stalk diameter was smaller for plants grown in the uneven distributions.

Variability of stalk diameter was increased in the uneven plant distributions. Under normal stand conditions the standard deviation was 2.54 mm, while the combined standard deviation for the variable stands was 3.11 mm. This corresponds to an increase of 0.57 mm for the irregular stands. The average coefficient of variation for plants grown in variable distributions was 2.73 percent larger than for the normal distribution. From these data it may be concluded that mean stalk diameter was 2.4 percent smaller while variability was significantly greater in the uneven stands.

TABLE 11.—*Effect of randomly variable plant distribution upon the stalk diameter of corn and its variability at maturity, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Percentage germination of seed planted	Seeds dropped per hill	Plants measured	Stalk diameter		
			Mean	Standard deviation	Coefficient of variation
	<i>Number</i>	<i>Number</i>	<i>Milli-meters</i>	<i>Milli-meters</i>	
75.....	4	1,024	23.23	2.94	12.66
60.....	5	968	23.46	3.20	13.64
50.....	6	1,042	22.74	3.16	13.90
Average *.....			23.14	3.11	13.44
100.....	3	1,001	23.72	2.54	10.71

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE NORMAL STAND

Percentage germination of seed planted	Stalk diameter					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	<i>Milli-meters</i>		<i>Milli-meters</i>			
75.....	-0.49	6.12	0.40	6.67	1.95	7.80
60.....	-.26	2.89	.66	11.00	2.93	11.27
50.....	-.98	10.89	.62	10.33	3.19	12.27
Average *.....	-.58	9.07	.57	11.40	2.73	13.65

* Refer to table 7, footnotes.

MATURITY

It is generally recognized that date of silking may be used as a measure of the relative time of maturity in corn. Silking notes were taken only on the plants grown in connection with the randomly variable stands. The data from this test are shown in table 12. In no instance was the number of days from planting to silking affected by plant distribution. The mean for plants resulting from seed germinating 100 percent was 68.68 days, while for plants grown in the variable distributions it was 68.79 days. In no case was there a significant increase or decrease in mean silking date or its variability.

TABLE 12.—*Effect of randomly variable plant distribution upon the length of interval from planting to silking and its variability, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Percentage germination of seed planted	Seeds dropped per hill	Plants measured	Length of interval		
			Mean	Standard deviation	Coefficient of variation
	<i>Number</i>	<i>Number</i>	<i>Days</i>	<i>Days</i>	
75.....	4	926	68.72	3.78	5.50
60.....	5	914	68.55	3.74	5.46
50.....	6	904	69.11	3.80	5.50
Average *.....			68.79	3.78	5.49
100.....	3	924	68.68	3.65	5.31

* Refer to table 7, footnotes.

TABLE 12.—*Effect of randomly variable plant distribution upon the length of interval from planting to silking and its variability, 1931—Continued*

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE NORMAL STAND

Percentage germination of seed planted	Length of interval					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Days		Days			
75	0 04	0 36	0 13	1 62	0 19	1.58
60	— .13	1.18	.09	1 12	.15	1.25
50	.43	3.91	.15	1.88	.19	1.58
Average ^a	.11	1.22	.13	1.86	.18	2 00

^a Refer to table 7, footnotes

GRAIN YIELD PER EAR-BEARING PLANT

Determinations of shelled-corn weight per ear-bearing plant for the systematically uneven distributions are reported in table 13. In no instance was there a significant difference in the means, although it is observed that they were slightly higher for the uneven than for the uniform stand. Uneven distribution, however, increased variability, as is indicated by an increase of 6.28 g in the combined standard deviation for uneven stands above the standard deviation for plants grown in uniformly 3-plant hills. However, the coefficient of variation, which was 5.15 percent greater for the uneven distributions, is not so suggestive of increased variability. Thus, according to the standard deviation, the increase in variability was statistically significant, whereas judged by the coefficient of variation it was not significant.

Table 14 presents the data for ears resulting from plants grown in the randomly variable distributions. As in the preceding data, significant differences in means were not found. In this instance, however, no induced variability was indicated by either the standard deviation or the coefficient of variation.

TABLE 13.—*Effect of systematically variable plant distribution upon the grain yield (moisture-free) of ear-bearing plants and its variability, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Type of stand	Distribution of plants in successive hills	Plants measured	Grain yield per plant		
			Mean	Standard deviation	Coefficient of variation
			Grams	Grams	
Uneven.....	2-4-2-4.....	Number 490	64.63	48.87	75.62
Do.....	1-3-5.....	487	68.74	52.43	76.27
Do.....	1-2-3-4-5.....	504	67.88	51.77	76.27
Average ^a			67.09	50.98	75.99
Uniform.....	3-3-3-3.....	500	63.10	44.70	70.84

^a Refer to table 7, footnotes.

TABLE 13.—*Effect of systematically variable plant distribution upon the grain yield (moisture-free) of ear-bearing plants and its variability, 1931—Continued*

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE UNIFORM STAND

Distribution of plants in successive hills	Grain yield per plant					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	<i>Grams</i>		<i>Grams</i>			
2-4-2-4.....	1.53	0.76	4.17	2.94	4.78	1.49
1-3-5.....	5.64	2.70	7.73	5.22	5.43	1.68
1-2-3-4-5.....	4.78	2.32	7.07	4.88	5.43	1.70
Average ^a	3.99	2.46	6.28	5.51	5.15	2.02

^a Refer to table 7, footnotes.TABLE 14.—*Effect of randomly variable plant distribution upon the grain yield (moisture-free) of ear-bearing plants and its variability, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Percentage germination of seed planted	Seeds dropped per hill	Plants measured	Grain yield per plant		
			Mean	Standard deviation	Coefficient of variation
	<i>Number</i>	<i>Number</i>	<i>Grams</i>	<i>Grams</i>	
75.....	4	749	76.36	46.77	61.25
60.....	5	748	75.58	46.62	61.68
50.....	6	765	74.11	46.18	62.31
Average ^a			75.34	46.57	61.81
100.....	3	748	73.44	44.82	61.03

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE NORMAL STAND

Percentage germination of seed planted	Grain yield per plant					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	<i>Grams</i>		<i>Grams</i>			
75.....	2.92	1.84	1.95	1.72	0.22	0.11
60.....	2.14	1.34	1.80	1.61	.65	.32
50.....	.67	.43	1.36	1.21	1.28	.64
Average ^a	1.90	1.48	1.75	1.92	.78	.48

^a Refer to table 7, footnotes.

PERFORMANCE OF PLANTS IN THE VARIOUS HILL TYPES

While the standard deviation is an absolute measure and the coefficient of variation a relative measure of variability, these constants do not fully reveal plant differences induced by the specific hill type.

Some interest attaches to an analysis of the plant development within the various kinds of hills classified according to the number of plants contained. It would seem that differences in the development and performance of plants growing in the various hill types may be attributed directly to the various degrees of plant competition within the hill resulting from stand inequalities. The results from the variable plant distribution studies in 1931 are summarized in this manner in tables 15 and 16.

TABLE 15.—*Performance of plants within each hill type of the systematically variable plant distributions, 1931*

UNIFORMLY 3 PLANTS							
Hill type (plants per hill)	Percentages of all plants, all ear-bearing plants, and all barren plants, that occurred in each hill type			Performance of plants within each hill type			
	Total plants	Ear-bearing plants	Barren plants	Mature plant height	Barren plants	Shelled corn *	
						Per plant	Per ear-bearing plant
				Inches	Percent	Grams	Grams
3	100 0	100 0	100 0	70 4	18 6	51 3	63 1
ALTERNATING 2 AND 4 PLANTS							
2	33 3	37 0	19.2	71 2	12 0	71 1	80 7
4	66 7	63.0	80 8	68 5	25.1	41 5	55.4
Mean.				69 5	20 7	51.4	61 6
ALTERNATING 1, 3, AND 5 PLANTS							
1	11 1	12 8	2 0	75 4	2.9	121.0	124.6
3	33 3	33 3	33.3	70.6	16 4	61.7	73 8
5	55 6	53.8	64 7	68.0	19 0	41 1	50.8
Mean.				69 7	16 4	56.8	68.7
ALTERNATING 1, 2, 3, 4, AND 5 PLANTS							
1	6.7	7 8	0.9	74.9	2.4	114 4	117 2
2	13 3	14 7	7 1	75 4	9 4	89 4	92.7
3	20.0	21 3	14.1	70.8	12.5	66.7	76 2
4	26 7	26 1	29 2	67.1	19 4	39.9	49.5
5	33 3	30 1	48 7	67 0	25.8	37 3	50.3
Mean.				69 5	17.7	56 0	67 9

* Moisture-free

In the case of the three different systematically variable stands (1) with alternating 2- and 4-plant hills, 33.3 and 66.7 percent of the plant population occurred in the respective hill types; (2) with alternating 1-, 3-, and 5-plant hills, 11.1, 33.3, and 55.6 percent of the plants were grown in the respective hill types; and (3) with alternating 1-, 2-, 3-, 4-, and 5-plant hills, 6.7, 13.3, 20.0, 26.7, and 33.3 percent of the plants occurred, respectively, in the various hill types.

TABLE 16.—Effect of randomly variable distribution of corn plants upon the performance of plants within each hill type of the various distributions, 1931

GERMINATING 100 PERCENT AND 3 KERNELS DROPPED PER HILL

Hill type (plants per hill)	Percentages of all plants, all ear-bearing plants, and all barren plants, that occurred in each hill type			Performance of plants within each hill type			
	Total plants	Ear-bearing plants	Barren plants	Mature plant height	Barren plants	Shelled corn *	
						Per plant	Per ear-bearing plant
				Inches	Percent	Grams	Grams
1.....	0.4	0.5	0	74.2	0	115.0	115.0
2.....	4.8	4.3	6.1	68.0	29.2	67.4	95.2
3.....	94.8	95.2	93.9	67.0	21.7	57.0	72.8
Mean.....				67.6	22.4	57.7	73.4

GERMINATING 75 PERCENT AND 4 KERNELS DROPPED PER HILL

1.....	2.4	2.6	1.9	70.8	20.0	84.7	105.8
2.....	15.0	15.9	12.3	69.7	20.6	79.6	100.3
3.....	41.4	41.4	41.4	66.6	25.2	57.0	76.2
4.....	41.2	40.1	41.4	64.8	27.2	46.2	63.5
Mean.....				66.8	26.6	56.6	76.4

GERMINATING 60 PERCENT AND 5 KERNELS DROPPED PER HILL

1.....	3.1	4.0	0	71.1	0	118.9	118.7
2.....	16.7	19.1	8.0	69.4	10.2	78.7	87.6
3.....	37.0	35.2	43.7	67.7	25.3	58.0	77.9
4.....	30.9	30.0	34.3	65.8	23.8	48.1	63.1
5.....	12.2	11.8	14.0	62.2	24.6	49.2	65.3
Mean.....				67.5	22.4	59.2	75.6

GERMINATING 50 PERCENT AND 6 KERNELS DROPPED PER HILL

1.....	3.3	4.2	9.4	71.0	2.9	103.9	107.0
2.....	16.0	19.0	7.3	69.2	11.6	98.0	99.5
3.....	29.0	29.0	29.1	67.2	25.6	54.0	72.6
4.....	30.2	27.7	37.4	66.3	31.6	45.0	65.8
5.....	18.4	17.9	20.0	62.5	27.6	45.5	62.9
6.....	3.1	2.1	5.8	63.8	48.5	24.7	48.0
Mean.....				66.7	27.1	55.9	74.1

* Moisture-free.

In the case of randomly variable stands resulting from the use of imperfectly germinating seed planted at an increased rate to compensate for dead seeds, the resulting 1-, 2-, and 3-plant hills from seed germinating 100 percent contained 0.4, 4.8, and 94.8 percent of the plant population, respectively. When seed corn germinating 75 percent was planted at 4 kernels per hill, 2.4, 15.0, 41.4, and 41.2 percent of the plants grew in 1-, 2-, 3-, and 4-plant hills, respectively. For seed corn germinating 60 percent and planted at 5 kernels per hill, the resulting percentages of the population occurring in 1-, 2-, 3-, 4-, and 5-plant hills were 3.1, 16.7, 37.0, 30.9, and 12.2 percent, respectively. And seed corn germinating 50 percent, planted at 6

kernels per hill resulted in hills containing 1, 2, 3, 4, 5, and 6 plants with 3.3, 16.0, 29.0, 30.2, 18.4, and 3.1 percent of the population within the respective hill types.

The data in tables 15 and 16 show essentially the same facts for both the systematically and randomly variable distributions: That the plants grown in 1- and 2-plant hills had relatively fewer barren plants, and conversely more plants bore ears than in hills containing 4, 5, and 6 plants, while 3-plant hills were intermediate. In every instance the mean height of mature plants grown in 1-plant hills was greater than for plants grown in 2-, 3-, 4-, 5-, or 6-plant hills, and this difference tended to become larger as the number of plants per hill increased. The mean weight of shelled corn per ear-bearing plant was greatest for hills containing only one plant and as the number of plants per hill increased the shelled-corn weight per plant tended to decrease rapidly. When the average weight of shelled corn for all plants was considered, the differences were more striking since the decreases for large hills were augmented by a higher number of barren plants.

In general, these data from uneven stands show the plant size and productivity to be in inverse relation to hill size.

DISCUSSION AND CONCLUSIONS

The irregular stands of dent corn under investigation were fully as variable as might ever be expected to occur in the field under practical farm conditions as a result of mechanical imperfections of the planter or the use of imperfectly germinating seed. The data are based on (1) three systematically variable stands, (2) three randomly variable stands, and (3) essentially uniform stands grown for comparison. All of these approximated an average of three plants per hill. The systematic distributions consisted of alternating hills with (1) 2 and 4 plants, (2) 1, 2, 3, 4, and 5 plants, and (3) 1, 3, and 5 plants, respectively. The randomly variable distributions were obtained by planting seed corn germinating 75, 60, and 50 percent at adjusted rates to average three sound seeds per hill.

It is shown that through application of the law of chance a very close estimation of the resulting distribution of viable seeds may be had before planting, when the germination percentage of the seed to be planted and number of kernels to be dropped per hill are known.

During a 14-year period, the systematically variable stands averaged 50 bushels per acre as compared with 49.9 bushels for the uniform stand. The most variable stand yielded 0.7 bushel or 1 percent less than the uniform stand.

In the unusually dry year of 1931, seed germinating 100, 75, 60, and 50 percent, planted at the respective rates of 3, 4, 5, and 6 seeds per hill, gave corresponding yields of 24.96 ± 0.65 , 25.50 ± 0.66 , 25.34 ± 0.66 , and 25.12 ± 0.65 bushels per acre. Differences between these are not statistically significant, and it is concluded that these stand irregularities have not affected yields.

The influence of these various stand irregularities upon the uniformity of plant development as measured by the standard deviation and coefficient of variation was determined in 1931 for the following characters: Plant height at several stages of development, ear height, stalk diameter, date of silking, and yield of grain per ear-bearing

plant. In nearly all cases the standard deviations and coefficients of variation were greater for the irregular stands, and frequently the increase was statistically significant though not large and of no great agronomic importance.

In the majority of cases the mean values for the various plant characters observed were slightly reduced by stand irregularity.

The effect of uneven stands upon the uniformity of plant development becomes more strikingly apparent if the plants grown within the various hill types are compared, since performance tended to vary inversely as the number of plants per hill. To illustrate: In the random distribution secured from corn germinating 50 percent, planted at 6 kernels per hill, the plants grown in 1-, 2-, 3-, 4-, 5-, and 6-plant hills yielded, respectively, approximately 107, 100, 73, 66, 63, and 48 g of moisture-free grain per ear-bearing plant. Similarly, the average mature height of plants grown in 6-plant hills was only 90 percent as great as for those grown in 1-plant hills, and the intervening hill types fell almost regularly between these extremes.

In general, it is evident that there may be considerable irregularity in the distribution of plants in a cornfield without the yield of grain per acre being materially affected. Such unevenness in stands, however, tends to increase the variability of plant development. Maximum uniformity among plants may be achieved through seed selection and planting practices that are conducive to uniformity of stand. Experience has indicated that stand irregularities materially greater than those herein considered, such as are sometimes caused by rodents, worms, birds, and soil washing, would undoubtedly increase the plant variability and lower the yield.

INFLUENCE OF PASTURE MANAGEMENT UPON THE GRAZING HABITS OF DAIRY CATTLE¹

By R. E. HODGSON

Agent, Bureau of Dairy Industry, United States Department of Agriculture; dairy husbandman, Western Washington Experiment Station; and assistant in dairy husbandry, Washington Agricultural Experiment Station

INTRODUCTION

Any improvement in pasture management that will cause lactating cows to obtain their sustenance with a minimum of grazing will conserve their energy for milk production. Energy expended in work incurred as a result of excess grazing is used at the expense of production, particularly in cases where herbage is sparse and supplemental barn feeding is not the general practice.

Few investigators have studied the physical activities of dairy cattle on pasture. Shepperd² made observations on three Hereford steers over a period of 24 hours, and reported that when on sweetclover pasture they averaged 8¼ hours' grazing, 7¼ hours' lying, 4 hours' sleeping, and 4½ hours' loafing (standing, not eating). Moseley, Stuart, and Graves³ reported that cows receiving pasture alone grazed on an average 9.16 hours per day, while animals receiving alfalfa in addition to pasture grazed on an average 6.50 hours, or 29 percent less. Cows receiving a limited-grain ration (1 pound of grain for every 6 pounds of milk produced) and cows receiving a full-grain ration (1 pound of grain for every 3 pounds of milk produced) grazed 39 and 42 percent less, respectively, than those on pasture alone. These observations were made between 5 a.m. and 9 p.m. on 2 days in July and on 2 days in August. Fuller⁴ concluded that cows kept in the barn during the winter stood approximately 50 percent of the time. As a result of studying the habits of 22 cows he found that approximately 3 hours a day was spent in eating.

The investigation described in this paper was undertaken to gain knowledge of the physical activities of lactating cows subjected to different types of grazing.

EXPERIMENTAL PROCEDURE

The data presented in this paper were obtained from an experiment at the Western Washington Experiment Station, conducted for the purpose of determining the efficiency of rotational grazing.⁵

Eight Holstein cows in about the same stage of lactation were divided into two lots as nearly alike as possible in respect to age, live weight, gestation, and production. Lot 1, consisting of four

¹ Received for publication Mar. 30, 1933; issued October 1933. Scientific paper no. 255, College of Agriculture and Experiment Station, State College of Washington.

² SHEPPERD, J. H. SWEET CLOVER EXPERIMENTS IN PASTURING. N. Dak. Agr. Expt. Sta. Bul. 211, 57 p., illus. 1927.

³ MOSELEY, T. W., STUART, D., and GRAVES, R. R. DAIRY WORK AT THE HUNTLEY FIELD STATION, HUNTLEY, MONT., 1916-1927. U.S. Dept. Agr. Tech. Bul. 116, 48 p., illus. 1929.

⁴ FULLER, J. M. SOME PHYSICAL AND PHYSIOLOGICAL ACTIVITIES OF DAIRY COWS UNDER CONDITIONS OF MODERN HERD MANAGEMENT. N.H. Agr. Expt. Sta. Tech. Bul. 35, 30 p., illus. 1923.

⁵ "Rotational grazing" is defined as restricted grazing of animals rotating over units of the entire area

animals, was subjected to continuous grazing⁶ on a 2-acre pasture. Grazing was maintained at the rate of two cows per acre during a grazing season of 147 days. Lot 2 was subjected to rotational grazing on a similar 2-acre pasture divided into six $\frac{1}{3}$ -acre plots. On each plot, grazing was maintained at the rate of 12 cows per acre. The carrying capacity of the rotation pasture was at the rate of two cows per acre for the 147 days. The frequency of rotation was governed by the rapidity with which the plots were grazed to a uniform height of about 1 inch.

Except for the method of grazing, both lots were managed identically. Each animal received a supplemental low-protein grain ration, at the rate of 1 pound for every 7 pounds of milk produced.

Records were kept of the length of time spent by each cow in grazing and lying and the number of times that grazing and lying occurred. Information was obtained for 5 different weeks at approximately monthly intervals, beginning April 28, May 28, June 27, July 30, and September 8. During the weeks of observation an attendant was on watch each day from 6 a.m. until dark. The cows were removed from the pasture at 4 a.m., at which time there were seldom any cows grazing. They were returned to the pasture at 6 a.m. During the afternoon milking they were off the pasture from 2 p.m. until 5 p.m. No data were taken after dark. Except during the week beginning September 8, all cows had discontinued grazing and were apparently settled for the night before the attendant left the field.

PRESENTATION OF RESULTS

Table 1 shows the amount of time spent in grazing and lying and the number of times that grazing and lying occurred for the individual cows subjected to the two types of grazing. The means are calculated from five different weekly observation periods made at approximately monthly intervals.

For cows subjected to continuous grazing the average time expended daily in grazing and lying was 439.10 minutes and 154.02 minutes, respectively, as compared with 411.08 minutes and 192.40 minutes, respectively, for cows subjected to rotational grazing. The cows subjected to rotational grazing obtained their sustenance in an average of 28.02 minutes less than did those grazing promiscuously over the large pasture. They were reclining on average 38.38 minutes more than cows on the open pasture.

The average number of times that grazing and lying occurred was slightly greater for the cows on the rotational-grazing system. The time expended in loafing (standing not eating) was 68.30 minutes per day for the cows on the rotation system, as compared with 82.88 minutes for the other cows. The shorter time expended in grazing and loafing and the increase in time of resting indicates that the cows subjected to rotational grazing were able to obtain their sustenance with a smaller expenditure of energy than those whose grazing was unrestricted.

Figure 1 depicts the activities of the grazing animals as the season advanced. The average number of minutes the cows were under observation per day varied with the time at which darkness set in. As the grazing season advanced the average time expended in grazing

⁶ "Continuous grazing" as used in this report refers to the unrestricted grazing of animals over the entire pasture area.

TABLE 1.—Average amount of time cows on continuously grazed pasture and on rotationally grazed pasture spent per day in grazing and in lying, and the number of times grazing and lying occurred

LOT 1. COWS ON CONTINUOUSLY GRAZED PASTURE

Cow no	Number of days under obser- vation	Observation (time per day (minutes)			Time spent in grazing per day (minutes)			Number of times grazing occurred per day			Time spent in lying per day* (minutes)			Number of times lying occurred per day		
		Mean	Prob- able error	Stand- ard de- viation	Mean	Prob- able error	Stand- ard de- viation	Mean	Prob- able error	Stand- ard de- viation	Mean	Prob- able error	Stand- ard de- viation	Mean	Prob- able error	Stand- ard de- viation
122	35	676.00	±1 004	8.80	432.50	±1 084	14.82	5.66	±0 304	2.67	151.75	±1 076	8.44	2.39	±0 083	0.66
126	35	676.00	±1 004	8.80	427.50	±1 365	11.97	7.34	±219	1.92	185.72	±1 924	10.15	2.93	±.195	1.53
134	35	676.00	±1 004	8.80	449.00	±1 460	12.81	8.20	±259	2.62	148.22	±1.125	8.82	2.14	±.011	.88
139	35	676.00	±1 004	8.80	445.35	±1 695	14.87	7.29	±219	1.92	130.35	±1 141	8.95	2.07	±.039	.304
Mean	-----	676.00	± 542	8.80	439.10	±.832	13.84	7.87	±.145	2.35	154.02	± 634	9.94	2.38	±.071	1.12

LOT 2. COWS ON ROTATIONALLY GRAZED PASTURE

Cow no	Number of days under obser- vation	Observation (time per day (minutes)			Time spent in grazing per day (minutes)			Number of times grazing occurred per day			Time spent in lying per day* (minutes)			Number of times lying occurred per day		
		Mean	Prob- able error	Stand- ard de- viation	Mean	Prob- able error	Stand- ard de- viation	Mean	Prob- able error	Stand- ard de- viation	Mean	Prob- able error	Stand- ard de- viation	Mean	Prob- able error	Stand- ard de- viation
214	35	671.78	±0 945	8.29	444.65	±0 978	8.58	8.03	±0 486	2.52	147.52	±1 740	13.65	2.18	±0 036	0.28
125	35	671.78	±.945	8.29	406.08	±1 083	9.59	10.66	± 425	3.73	188.40	±1 373	10.77	3.29	±.080	1.25
142	35	671.78	±.945	8.29	407.50	±1.114	9.77	7.80	±205	1.80	207.15	±1 399	10.97	3.54	±.129	2.03
146	35	671.78	±.945	8.29	390.50	±.931	8.17	9.46	± 310	2.72	226.78	±1 297	10.17	3.50	±.088	1.38
Mean	-----	671.78	± 511	8.29	411.08	± 610	9.89	8.83	±.150	2.44	192.40	± 823	12.91	3.13	±.098	1.54

* Based on 28 days of observation.

by the cows on the rotationally grazed pasture decreased from 414 minutes per day during the week of April 28 to 385.8 minutes during the week of September 8. During the same period the average grazing time per day for the cows on the open pasture increased from

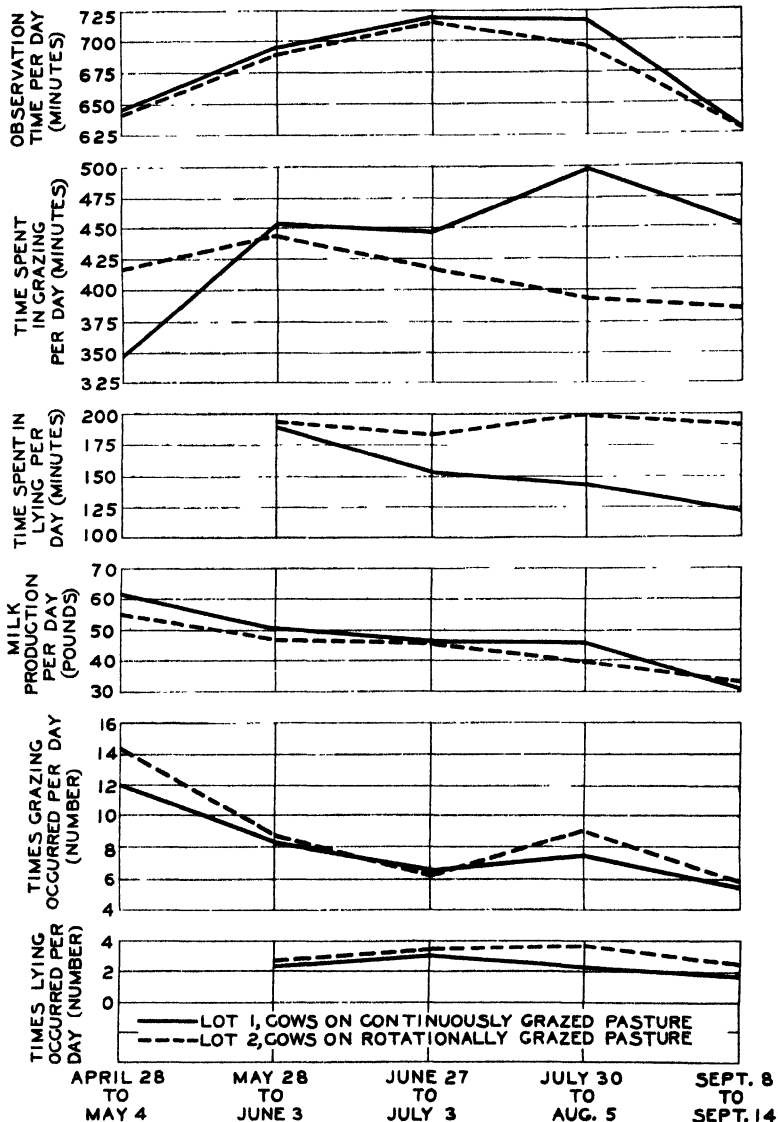


FIGURE 1.—Effect of advancement in season upon the grazing and lying activities of lactating cows.

339.28 minutes to 455.35 minutes. The greater amount of time spent in eating by the cows on rotationally grazed pasture during the first week of observation may have been the result of their grazing over two plots in succession, whereas the other group had access to an abundance of grass throughout the period.

From the week beginning May 28 to the week of September 8, the decrease in the average time spent in lying was 66.98 minutes for the cows on the continuously grazed pasture as compared with 8.05 minutes for the cows on the rotationally grazed pasture. The number of times that lying occurred also decreased more in the case of the cows grazing unrestrictedly. The trend of milk production remained practically the same for the two lots throughout the season.

As the season advanced the cows on the rotational-grazing system expended less energy in grazing. This did not cause a significant increase in their milk production, but it did result in a greater gain in live weight as compared with that of the cows grazing over the open pasture.

Intensive systems of pasture management require that rotation units be closely grazed in order that the herbage may be kept succulent and in a highly nutritious condition. It is difficult, however, to determine the optimum number of days that milking cows should be maintained on a rotation unit in order to accomplish uniform close grazing without decrease in live weight or milk production. Table 2 shows the effect of increasing the number of days that cows are maintained on a rotation plot on their grazing and lying activities. Observations were made while cows were maintained on individual plots for 1, 2, 3, and 4 days. The cows grazed on an average 1.8 percent more of the total time observed on the fourth day than on the first.

Table 3 shows the effect on live weight of increasing the number of days that cows are maintained on a rotation plot. Individual live weights of the four cows in lot 2 were recorded over a period of 45 consecutive days, 15 of which were taken at the end of the first day on a rotation plot, 15 at the end of the second day, and 13 at the end of the third day. There was a significant relation between loss in live weight and increasing days on a rotation plot. This is evidenced by the average live weight loss of 10.49 pounds the second day and 14.59 pounds the third day as compared with the first day.

Table 4 shows the effect on milk production of increasing the number of days that cows are maintained on a rotation plot. A comparison between milk production and the number of days that cows were maintained on a plot was made for the 1931 and 1932 seasons.

The evening milk production (the last milking of the experimental day) and the total day's production (including the evening milking plus the following morning milking) were used in making this comparison.

The data show that milk production decreased as the number of days that cows were maintained on a rotation unit increased. This relationship was more pronounced during the 1932 season when cows were maintained on individual plots for as many as 4 days. Reduction in the amount of milk produced as a result of increasing the number of days that the cows were on a rotation unit was evidenced in both the evening milking and the total day's production.

TABLE 3.—*Effect of increasing the number of days that cows are maintained on a rotation plot on their average live weight*

Cow no	Weight (pounds) first day on plot ^a			Weight (pounds) second day on plot ^a			Decrease	Weight (pounds) third day on plot ^b			Decrease
	Mean	Probable error	Standard deviation	Mean	Probable error	Standard deviation		Mean	Probable error	Standard deviation	
214	1,305 00	±1 189	6.83	1,289 00	±1 227	7.33	16 00	1,286 54	±1 500	8.02	18 46
125	1,413 67	±1 062	6.10	1,406 33	±1 062	6.10	7 34	1,406 54	± 980	5 21	7 13
142	1,164 93	± 994	5.71	1,156 33	±1 170	6.72	8 60	1,150 38	± 660	3 53	14 55
146	1,221 67	± 869	4.99	1,211 67	±1 057	6.07	10 00	1,203 46	±1 109	5 93	18 21
Average	1,276 32	±1 028	5.91	1,265 83	±1 129	6.56	10 49	1,261 73	±1 062	5.68	14 59

^a Data are based on 15 days.^b Data are based on 13 days.

The effect of increasing the number of grazing days per plot upon the physical activities, live weight, and production of lactating cows illustrates the disadvantage of requiring heavy-milking cows to graze the area uniformly close before they are moved to the next unit of the rotation series. It also indicates the advisability of allowing the heavy-milking cows to graze the choicest portion of the grass, to be followed by dry stock and heifers that must obtain their sustenance by close grazing.

TABLE 4 *Effect on milk production of increasing the number of days that cows are maintained on a rotation plot, 1931 and 1932*

Year and items of comparison	Number of cow-days	Milk yield (pounds) first day on plot			Number of cow-days	Milk yield (pounds) second day on plot			Variation from first day (percent)
		Mean	Probable error	Standard deviation		Mean	Probable error	Standard deviation	
1931									
Milk production									
Evening milking									
Apr. 30 to July 2	119	20 52	± 0 298	4 82	106	19 57	± 0 318	4.76	-4 63
July 3 to Sept. 9	91	14 08	± 279	3 95	96	14 02	± 286	4.15	-1 43
Average	105	17 30	± 288	4 38	101	16 80	± 302	4.46	-2.89
Evening and morning milking									
Apr. 30 to July 2	119	41 16	± 567	9 17	106	38 36	± 610	9 31	-6.80
July 3 to Sept. 9	91	28 88	± 549	7.77	96	27 94	± 600	8.24	-3.25
Average	105	35.02	± 558	8.47	101	33 15	± 605	8.78	-5.34
1932									
Milk production									
Evening milking									
Apr. 28 to July 5	88	23 27	± 232	3.22	88	23 05	± 227	3 15	- .95
July 6 to Sept. 13	96	17 54	± 164	2 38	92	17.39	± 160	2.28	-1.86
Average	92	20 40	± 189	2.80	90	20.22	± 194	2.72	-1.88
Evening and morning milking									
Apr. 28 to July 5	88	51.10	± 535	7.17	88	48.52	± 522	7.00	-5.05
July 6 to Sept. 13	96	38 20	± 324	4.71	92	36 68	± 334	4.69	-3.98
Average	92	44.65	± 430	5.94	90	42.60	± 428	5.84	-4 50

^a Includes the evening milk production on the day in question plus the following morning's production.

TABLE 4.—*Effect on milk production of increasing the number of days that cows are maintained on a rotation plot, 1931 and 1932—Continued*

Year and items of comparison	Number of cow-days	Milk yield (pounds) third day on plot				Variation from first day (percent)	Number of cow-days	Milk yield (pounds) fourth day on plot				Variation from first day (percent)
		Mean	Probable error	Standard deviation	Mean			Probable error	Standard deviation			
1931												
Milk production												
Evening milking -												
Apr 30 to July 2	48	20 04	±0 301	3 09	-2 34							
July 3 to Sept 9	30	13 73	± 514	4 18	-2 49							
Average	39	16 88	± 408	3 64	-2 43							
Evening and morning milking ^a												
Apr 30 to July 2	48	40 21	± 818	8 41	-2 31							
July 3 to Sept 9	30	28 86	± 1 172	9 52	- 07							
Average	39	34 54	± 995	8 96	-1 37							
1932												
Milk production												
Evening milking -												
Apr 28 to July 5	68	21 85	± 262	3 21	-6 10	32	22 44	±0 371	3 11	- 3 57		
July 6 to Sept 13	80	16 52	± 159	2 11	-5 82	12	15 67	± 316	1 56	-10 66		
Average	74	19 18	± 210	2 66	-5 98	22	19 06	± 344	2 34	-6 57		
Evening and morning milking ^a												
Apr 28 to July 5	68	48 71	± 581	6 91	-4 62	32	48 50	± 887	6 67	-5 09		
July 6 to Sept 13	80	35 35	± 346	4 58	-7 46	12	34 31	± 724	3 76	-10 10		
Average	74	42 04	± 464	5 74	-5 84	22	41 42	± 806	5 22	- 7 23		

^aIncludes the evening milk production on the day in question plus the following morning's production

CONCLUSION

Data are presented which show the comparative grazing habits of cows subjected to two types of pasture management.

Cows subjected to rotational grazing, on an average grazed 28.02 minutes less and spent 38.38 minutes more in lying than similar cows on an open pasture where unrestricted grazing occurred. Cows on the rotational system expended an average of 411.08 minutes daily in grazing and 192.40 minutes in lying. The average number of times that grazing and lying occurred in this lot was 8.83 and 3.13, respectively, as compared with 7.87 and 2.38 for those on the continuously grazed pasture.

As the grazing season advanced the cows on the rotational system expended less time in grazing and more in lying, whereas those on the continuously grazed pasture spent more time in grazing and less in lying.

Increasing the number of days that cows were maintained on a rotation unit effected a marked increase in the number of times that grazing occurred but did not materially increase the average time expended in grazing. Cows that grazed on a plot for 4 days were lying on an average 25.50 percent less on the fourth day than on the first.

A decrease in live weight accompanied an increase in the number of days that the cows were maintained on a pasture unit. The average decrease at the end of the second and the third day was 10.49 pounds and 14.59 pounds, respectively.

There was a significant decrease in milk production, which corresponded with an increase in the number of days that the cows were maintained on a rotation unit.

TRUE AND APPARENT DIGESTIBILITY OF OAT HULLS AND ALFALFA MEAL BY SWINE, WITH SPECIAL REFERENCE TO THE ABILITY OF SWINE TO DIGEST CELLULOSE AND CRUDE FIBER¹

By H. H. MITCHELL, *chief in animal nutrition*, and T. S. HAMILTON, *associate in animal nutrition*, Department of Animal Husbandry, Illinois Agricultural Experiment Station

INTRODUCTION

Although oat hulls are used to a considerable extent in swine feeding, as a constituent part either of the whole oat grain, or of oat feed, or of commercial mixed feeds, their digestibility by swine has never been determined, insofar as the writers are aware. They are not ordinarily accorded any high feeding value, mainly because of their high content of crude fiber. Also, no determinations of the digestibility of alfalfa hay by swine appear to have been reported in the literature.

The ability of swine to digest the crude fiber of feeds has been variously reported, the coefficients of digestion ranging from 10 to 90. This is probably due to differences in composition of the constituents making up the crude fiber of the various feeds, as well as to differences in the method of analysis for crude fiber. It is commonly supposed that lignocelluloses are not appreciably digested by swine, or by other animals with a digestive tract affording no opportunity for the stagnation and extensive fermentative decomposition of feed. It seems reasonable to expect, on the other hand, that the hemicelluloses, found to a variable extent in the crude fiber, will be broken down more readily by the intestinal bacteria than any other component of this fraction of carbohydrates. It would seem, however, that these more soluble constituents in crude fiber may be responsible for coefficients of digestion possessing a purely fictitious significance. If, during their passage through the digestive tract, they become more soluble in the acid and alkali used in the crude-fiber determination, because of the prolonged action of the slight acidity of the gastric juice, or possibly to a slight but incomplete disintegration by bacteria, they will be included in the nitrogen-free extract of the feces rather than in the crude-fiber fraction to which they originally belonged. This change in classification would occasion an increase in the estimated digestibility of crude fiber and a decrease in that of nitrogen-free extract, both effects of course having no biological significance. Possibly all digestion coefficients for the crude fiber of feeds are more or less high, because of this change in solubility of some of the compounds included therein during their passage through the intestinal tract of an animal.

REVIEW OF LITERATURE

The published evidence concerning the digestibility of the true celluloses of feeds by swine has been interpreted to mean that swine are not inferior to ruminants in this respect. The evidence, however,

¹ Received for publication May 8, 1933; issued October 1933.

is not impressive. In 1913 Fingerling and his associates (2) ² reported the results of a series of digestion trials with 2 wethers and 2 pigs, an alkali-digested straw pulp, immature grass, and wheat chaff being used as sources of cellulose. The average digestibility of crude fiber in the straw pulp in the case of the pigs was almost 95 percent and was appreciably higher than the coefficients obtained in the trials with the wethers. The crude fiber of the other feeds was digested much better by the wethers than by the pigs. The authors explain the high digestibility of the crude fiber of straw pulp by pigs on the assumption that it was practically pure cellulose, while the crude fiber of the grass and the wheat chaff was lignified to a great extent.

Similarly Woodman and his associates studied the digestibility of sugar beets (18), and of sugar-beet pulp (17), using two pigs in each trial. Average digestion coefficients of 90 and 84, respectively, were obtained for the crude fiber. Commenting on these results, the authors (17, p. 660) say:

The capacity of pigs to deal with fibre is usually regarded as very limited. The present results, however, show that this capacity is dependent on the nature of the fibre in the feedingstuff, and that when the fiber is non-lignified (that is, when it is composed mainly of cellulose) then such fibre may be digested to a very high degree.

The difficulty in interpreting both of these experiments in terms of cellulose is the uncertainty that the crude fiber either of the treated straw pulp or of the sugar-beet pulp is cellulose. In the first case, the alkali treatment may have initiated hydrolytic changes in the cellulose that would make it more amenable both to bacterial fermentation and to further hydrolytic cleavages in the intestinal tract of the pig, so that it would, in large part, pass from the crude-fiber constituents of the feed to the nitrogen-free extractives of the feces. In the second case, the crude fiber of the sugar-beet pulp may consist largely of the more difficultly soluble hemicelluloses rather than of cellulose, for all that is known to the contrary.

The majority of experiments concerned with the digestion of cellulose by other animals than the pig that possess a simple digestive tract indicate a very inconsiderable digestibility. Reference may be made in particular to the work of Scheunert and Löttsch (15) and of Thomas and his associates (16) on the dog, the work of Von Knierem (9) and Henning (6) on poultry, and of Fölling (3) on men.

EXPERIMENTAL PROCEDURE

The primary purpose of the experiment reported in this paper was the determination of the true digestibility of oat hulls by swine. This was accomplished in two series of digestion trials. In the first trials, the ration fed contained 30 percent of oat hulls and 70 percent of other constituents presumably completely digestible, i.e., 62 percent of cornstarch, 5 percent of sucrose, and 3 percent of a mineral mixture that contained the following ingredients: Steamed bone meal, 30 percent; ground limestone, 30 percent; NaCl, 30 percent; MgCO₃, 3 percent; K₂CO₃, 3 percent; K₂SO₄, 2 percent; FeCl₂, 1.5 percent; and KI, 0.5 percent.

In the second series of trials the ration contained, in place of the oat hulls, a mixture of cellulose and starch, the former equalling the

² Reference is made by number (italic) to Literature Cited, p. 434.

crude-fiber content of 30 percent of oat hulls. This ration contained 83.95 percent of starch, 8.05 percent of dried paper pulp (cellomass) or cellophane, 5 percent of sucrose, and 3 percent of the same mineral mixture. The purpose of the second ration, fed in the same amounts as the first, was to measure the metabolic products in the feces, which are presumably (11, *p.* 21) related to the intake of dry matter and to its content of crude fiber.

The experimental animals were 4 barrows, whose weights averaged 168, 175, 178, and 185 pounds respectively, for the entire period of the digestion trials. The 2 lightest pigs belonged to the Duroc-Jersey breed, and the 2 heaviest belonged to the Poland China breed. The pigs were practically constant in weight throughout the experiment.

The collection periods were 10 days long and were preceded by preliminary periods of at least 4 days, during which the same amount and kind of feed was consumed. All animals in all the periods consumed 1,200 g daily of the experimental rations in two equal portions given in the morning and late afternoon. There was no refused feed at any time.

A single digestion period on alfalfa meal was completed on 1 pig only, at the end of the 2 experimental periods described above, 3 of the pigs refusing to consume the alfalfa ration in sufficient amounts. The alfalfa-meal ration was patterned after the oat-hull ration, the substitution of one feed for the other being made in such a way that the crude-fiber content of the rations would be approximately the same.

The feed, feces, and urine were analyzed by the methods of the Association of Official Agricultural Chemists (1), except for crude fiber, which was determined by a modification of a method devised by Forbes and his associates (4). Since the digestibility of crude fiber is of primary interest to this study, the method used for its determination is given in detail.

A sample of feed or feces of 1½ to 3 g, depending on the crude-fiber content, was weighed out, 200 cc of 1.25 percent boiling sulphuric acid was added, and the mixture boiled for 30 minutes under a reflux condenser. Then 200 cc of 3.52 percent boiling sodium hydroxide solution was added and the mixture again boiled for 30 minutes. It was filtered on a Buchner funnel through fine linen (tracing cloth), moderate suction being used if necessary, and it was washed with hot water until the washings were free of chlorides. The residue on the linen was transferred to a beaker with water and filtered through a Gooch crucible provided with a pad of asbestos. The crucible and contents were dried by heating in an oven overnight at a temperature of 105° C., weighed, ignited, and weighed again, the difference in weights representing the crude fiber.

In the writers' experience there was no advantage in extracting the dried crude fiber with fat solvents. Apparently, in all ordinary samples at least, the fat in the sample is completely saponified during the 30-minute treatment with acid and alkali.

EXPERIMENTAL RESULTS WITH OAT HULLS

The oat hulls used in this experiment were prepared from whole oats by the Division of Swine Husbandry through the courtesy of Dr. W. E. Carroll. Care was taken to exclude practically all unhulled

kernels. The chemical analysis of the oat hulls gave the following results: Moisture, 8.05 percent; crude protein, 4.52 percent; ether extract, 3.28 percent; ash, 7.20 percent; crude fiber, 26.82 percent; and nitrogen-free extract, 50.13 percent. The gross-energy content, determined with the bomb calorimeter, was 3.98 calories per gram.

The coefficients of apparent digestibility of the oat-hull ration, as determined on the ordinary assumption that all the constituents of the feces are indigestible residues of the feed, are given in table 1.

TABLE 1.—Coefficients of apparent digestibility of the oat-hull ration when eaten by swine

Pig no.	Coefficients of apparent digestibility					
	Dry matter	Crude protein	Nitrogen-free extract	Crude fiber	Ether extract	Gross energy
2-PC	74.4	49.8	85.0	10.3	64.7	74.2
3-DJ	74.3	48.0	85.1	8.8	60.0	73.8
4-PC	75.6	47.5	85.9	14.7	66.6	75.0
5-DJ	76.0	46.4	86.6	15.6	48.3	75.6

When the nitrogen-free rations were made up for the second series of digestion trials, it was assumed that both the paper pulp (cello-mass) and the cellophane, serving as crude fiber, consisted entirely of cellulose. On analysis of the rations, however, it was found that while the cello-mass ration contained 7.47 percent of crude fiber, the cellophane ration contained only 4.12 percent. The cellophane itself was found to contain only 55.20 percent of crude fiber. The remainder of the cellophane, as the writers have since found, consists mainly of glycerol.

The fecal constituents excreted while the pigs were on the low-nitrogen rations (which contained about 0.05 percent of nitrogen) were assumed to consist, except for the crude fiber, entirely of the so-called metabolic products originating from the body rather than from the food. The metabolic dry substance (excluding the crude fiber), the metabolic nitrogen, and the metabolic ether extract excreted per kilogram of dry matter consumed, are given in table 2 for each pig.

TABLE 2.—Metabolic products excreted in the feces of swine per kilogram of dry matter consumed

Pig no.	Dry matter	Nitrogen	Ether extract	Pig no.	Dry matter	Nitrogen	Ether extract
	Grams	Grams	Grams		Grams	Grams	Grams
2-PC	32.9	0.81	1.00	5-DJ	32.2	0.67	1.03
3-DJ	32.2	.75	1.67				
4-PC	35.3	.80	2.26	Average	33.1	.76	1.49

The amounts of crude fiber excreted when the pigs were on these rations were very nearly the same as the amounts of crude fiber ingested. Thus, for the two pigs on the cello-mass ration, nos. 2-PC and 3-DJ, 89.6 g of crude fiber were consumed daily, and 81.4 and

86.6 g, respectively, were excreted in the feces. For the two pigs on the cellophane ration, nos. 4-PC and 5-DJ, 49.4 g of crude fiber were consumed daily and 54.4 and 48.6 g, respectively, were determined in the feces. The average coefficient of digestibility was only 3.5 percent.

The computation of the true digestibility of the oat hulls is given in table 3, which contains the pertinent data for pig 2-PC.

TABLE 3.—*Computation of the true digestibility of oat hulls for pig 2-PC*

[All weights are in grams per day and the energy is in calories per day]

Items	Crude protein	Nitrogen-free extract	Crude fiber	Ether extract	Gross energy
Nutrients in 360 g of oat hulls.....	16 27	180 5	96 5	11 81	1, 431
Nutrients in feces.....	10 25	132 6	98 0	3 81	1, 137
Nutrients in metabolic products.....	5 51	17 0	—	1 09	115
Undigested nutrients of oat hulls.....	4 74	115 6	98 0	2 72	1, 022
Digested nutrients of oat hulls.....	11 53	64 9	0	9 09	409
Coefficients of true digestibility.....	70 9	36 0	0	77 0	28 6

In the first line of figures are given the weights of nutrients contained in 360 g of oat hulls, representing 30 percent of the 1,200 g of ration consumed. In the second line are the weights of constituents in the feces excreted per day. On the assumption that all other components of the ration but the oat hulls are completely digestible, these fecal constituents include (1) the undigested material from 360 g of oat hulls, and (2) the metabolic products produced by 1,200 g of the ration. In the third line of figures are the amounts of fecal constituents (after making certain corrections) passed daily while the pig was receiving 1,200 g of the nitrogen-free diet. The crude fiber excreted when the pigs were on this diet is not included, since it was assumed to represent undigested crude fiber and in fact approximated closely the amounts of crude fiber consumed. Small adjustments (the correction above referred to) were made for all constituents to allow for small differences in the dry-matter content of 1,200 g of the two rations. These values (third line) are taken to measure the excretions of metabolic constituents due to the consumption of 1,200 g of the oat-hull ration. Hence, the differences between the values in the second line and those in the third are estimates of the indigestible constituents from the 360 g of oat hulls consumed. These differences make up the fourth line of figures in table 3. Deducting the values in the fourth line (undigested nutrients of oat hulls) from the corresponding values in the first line (nutrients contained in oat hulls) gives the nutrients digested from the oat hulls (fifth line), and these, expressed as a percentage of the amounts of nutrients contained in the oat hulls, are the coefficients of true digestibility, given in the last line of the table.

The coefficients of true digestibility of oat hulls for the four pigs, computed as shown in table 3, are included in table 4. These coefficients represent the nutrients actually absorbed from the digestion of oat hulls. An average of 66 percent of the crude protein and only 30 percent of the gross energy is utilized in digestion. For 100 pounds of oat hulls there appear to be only some 29 pounds of total digestible nutrients and 2.99 pounds of digestible protein.

TABLE 4.—Coefficients of true digestibility of oat hulls when eaten by swine

Pig no.	Coefficients of true digestibility					Total digestible nutrients per 100 pounds of oat hulls
	Crude protein	Nitrogen-free extract	Crude fiber	Ether extract	Gross energy	
2-PC.....	70.9	36.0	0	77.0	28.6	Pounds 26.9
3-DJ.....	66.2	36.0	0	78.7	27.6	26.9
4-PC.....	67.2	41.9	3.5	89.9	32.6	31.6
5-DJ.....	60.4	44.4	4.5	62.2	32.7	30.8
Average.....	66.2	39.6	2.0	76.9	30.4	29.0

For all practical purposes even these values are too high, since it is customary, and logical, to charge against a feed the metabolic fecal material lost during its digestion. This charge is included in the ordinary coefficients of (apparent) digestibility. In the present experiments, such coefficients may be obtained by adding to the estimated undigested residue of each nutrient 30 percent of the estimated metabolic excretion of the same nutrient (since the ration contained 30 percent of oat hulls), subtracting the sum from the amount of the nutrient contained in 360 g of oat hulls, dividing the difference by the latter amount, and multiplying by 100.

An illustration of the calculation may be given again from the data for pig 2-PC contained in table 3. The calculation of the coefficient of apparent digestibility for protein would be $\frac{16.27 - (4.74 + 1.65)}{16.27} \times 100 = 60.7$, 1.65 being 5.51×0.30 . A summary of the coefficients of apparent digestibility of oat hulls for the four pigs is given in table 5.

TABLE 5.—Coefficients of apparent digestibility of oat hulls when eaten by swine

Pig no.	Coefficients of apparent digestibility					Total digestible nutrients in 100 pounds *
	Crude protein	Nitrogen-free extract	Crude fiber	Ether extract	Gross energy	
2-PC.....	60.7	33.1	0	74.2	26.2	Pounds 24.8
3-DJ.....	56.8	33.3	0	74.2	25.1	24.7
4-PC.....	57.3	38.6	3.5	83.7	29.8	29.0
5-DJ.....	52.1	41.4	4.5	59.4	30.3	28.7
Average.....	56.7	36.6	2.0	72.9	27.8	26.8

* Computed on the basis of the composition of oat hulls used in this investigation.

By applying these digestion coefficients to the chemical composition of the oat hulls, it appears that each 100 pounds contain 2.56 pounds of digestible crude protein, 18.35 pounds of digestible nitrogen-free extract, 0.54 pounds of digestible crude fiber, 2.39 pounds of digestible ether extract, and 26.83 pounds of total digestible nutrients.

The digestibility of oat hulls by swine may be compared with its digestibility by other classes of livestock insofar as such determinations have been made. A compilation of such determinations is given in table 6.

TABLE 6. —*Comparison of the digestibility of oat hulls by different species of farm animals*

Kind of animal	Animals in test	Digestion coefficients				Total digestible nutrients in 100 ^a pounds	Literature reference
		Crude protein	Nitrogen-free extract	Crude fiber	Ether extract		
	Number					Pounds	
Swine	4	57	37	2	73	27	
Sheep	2	12	33	50	14	32	(10)
Do ^b		39	59	56	77	52	(5)
Do	1	41	33	44	70	35	(8)
Horse	2	100	11	22	82	22	(10)

^a Based on analysis of oat hulls given in this paper

^b Oat-hull clippings

The great discrepancy among the results for sheep precludes the possibility of any comparison of this species with swine with respect to their ability to digest oat hulls. It is apparent, however, that the pig is greatly inferior to both the sheep and the horse in its ability to digest the crude fiber of oat hulls.

It will be noted, both in the oat-hulls experiments and the alfalfa-meal experiment described later, that the crude fiber of the corresponding rations was apparently digested somewhat better than that of the oat hulls or the alfalfa meal themselves, although these feeds presumably were the sole source of crude fiber in their respective rations. This was due to the fact that the rations in each case contained slightly more crude fiber than would be predicted from their content of oat hulls or alfalfa meal and the composition of these feeds. All the crude-fiber determinations, performed in triplicate, gave results agreeing satisfactorily. The discrepancy between the analysis of the feeds and of the rations may have been the result either of an imperfect mixing of the rations, so that somewhat nonrepresentative samples were obtained for chemical analysis, or of an interference of the starch and sugar with the crude-fiber determination. In either case, more confidence may be felt in the crude-fiber determinations of the feeds than of the rations. It is assumed that the constituents of the rations were properly weighed out.

The crude fiber of oat hulls is known to contain some pentosans (14) and considerable lignin (13) equivalent to 5.6 to 6.9 percent of the dried hulls.

During these digestion trials the urine as well as the feces was collected and was analyzed for nitrogen. Its gross energy content was also determined, a Parr oxygen bomb calorimeter being used for the purpose. The results of these determinations are given in table 7, together with the intake of nitrogen and energy and the nitrogen balances.

TABLE 7. --Daily metabolism of nitrogen and energy on the oat-hull ration and the low-nitrogen rations when eaten by swine

Pig no.	Metabolism of—	Oat-hull ration				Low-nitrogen rations			
		Intake	Feces	Urine	Balance	Intake	Feces	Urine	Balance
2-PC	Nitrogen.....grams	3 26	1.64	2 89	-1.27	0 58	0 87	2.45	-2.74
	Energy.....calories	4,408	1,137	58	-----	4,336	439	40	-----
3-DJ	Nitrogen.....grams	3 26	1 70	1 21	+ 35	.58	.81	1 84	-2.07
	Energy.....calories	4,408	1,156	24	-----	4,336	466	34	-----
4-PC	Nitrogen.....grams	3 26	1 71	2 59	-1.04	.66	.86	2 71	-2.91
	Energy.....calories	4,408	1,100	56	-----	4,252	355	47	-----
5-DJ	Nitrogen.....grams	3 26	1.84	2 75	-1.33	.66	.72	2.66	-2.72
	Energy.....calories	4,408	1,075	48	-----	4,252	307	50	-----

The fact that the urinary nitrogen of a pig on the oat-hull ration did not show a consistent increase over that for the same pig while on the low-nitrogen ration, indicates a very nearly complete utilization of the absorbed nitrogen of the oat hulls in replacing the endogenous losses of nitrogen. The intake of oat-hull nitrogen was insufficient, however, to establish equilibrium. The urinary-nitrogen excretions per day on the low-nitrogen rations, expressed in milligrams per kilogram of body weight, were 30.2, 23.0, 32.2, and 34.5, respectively. These are very close to the lowest observed endogenous nitrogen output of the pig (12, p. 9).

The gross energy of the urine was also about the same on the two rations. By assigning a proportionate share of this energy to the oat hulls, it may be estimated that the metabolizable energy averages 1,166 calories per kilogram of dry matter.

Oat feed, consisting largely of oat hulls, has been compared in feeding value with wheat bran in the feeding of dairy cows. For dairy cows oat feed is said to be worth 70 percent as much as wheat bran, in amounts not to exceed one fourth of the grain ration (1a, p. 15). For swine, oat hulls are worth much less than half as much as wheat bran. On the basis of average analyses for the two feeds and reported digestion coefficients for wheat bran (7) and those obtained here for oat hulls, wheat bran contains 58.3 pounds of total digestible nutrients and 12.0 pounds of digestible crude protein per 100 pounds, while oat hulls contain 24.8 pounds of total digestible nutrients and 2.3 pounds of digestible crude protein per 100 pounds.

EXPERIMENTAL RESULTS WITH ALFALFA MEAL

At the conclusion of the metabolism experiment involving the use of the low-nitrogen rations, an attempt was made to secure information concerning the digestibility of alfalfa hay. A ration similar to the oat-hull ration, but containing alfalfa hay in an amount to furnish the same percentage of crude fiber, was given the four pigs, the transition from the low-nitrogen rations being made gradually. However, only one of the pigs, no. 3-DJ, could be induced to consume 1,200 g of this ration daily, the amount in which the other rations were fed. Hence, the experiment on alfalfa hay was confined to this pig.

The alfalfa meal used in this test contained 10.50 percent of water, 13.44 percent of crude protein, 2.16 percent of ether-soluble material, 7.61 percent of ash, 29.26 percent of crude fiber, and 37.03 percent of

nitrogen-free extract. Its gross energy content was equivalent to 3.95 calories per gram. The ration in which this feed was fed contained 27.51 percent of alfalfa meal, 3.00 percent of the same mineral mixture used in the other rations, 5.00 percent of sucrose, and 64.49 percent of cornstarch. The same plan of feeding and length of feeding period were used as in the two preceding experimental periods.

The coefficients of apparent digestibility of the alfalfa ration for the one pig were: 79.7 for dry matter, 37.5 for crude protein, 94.0 for nitrogen-free extract, 10.8 for crude fiber, and 78.5 for gross energy. The ether extract contained in the feces (10.53 daily) exceeded that contained in the feed (7.80 daily). By calculating the digestibility of the alfalfa meal by the same methods that were used for oat hulls, the following coefficients of true digestibility were obtained: 45.9 for crude protein, 71.1 for nitrogen-free extract, 1.9 for crude fiber, and 38.6 for gross energy. Again, the digestibility of the ether extract could not be computed. According to these results, applied to the composition of the alfalfa meal used, there were 33.0 pounds of total digestible nutrients per 100 pounds of alfalfa meal.

The apparent digestibility of the alfalfa meal in the above ration is represented by the following coefficients: 42.7 for protein, 67.4 for nitrogen-free extract, 1.8 for crude fiber, and 36.1 for energy. The total digestible nutrients amounted to 31.3 pounds per 100 pounds of alfalfa meal.

The metabolism of nitrogen and energy of pig 3-DJ on the alfalfa ration and the nitrogen-free ration is summarized in table 8.

TABLE 8. --*Daily metabolism of nitrogen and of energy for pig 3-DJ on the alfalfa-meal ration and the low-nitrogen ration*

Metabolism of	Alfalfa ration				Low-nitrogen ration			
	Intake	Feces	Urine	Balance	Intake	Feces	Urine	Balance
Nitrogen grams	7 45	4 66	2 48	+ 31	0 58	0 81	1 84	- 2 07
Energy calories	4,273	920	87		4,336	466	34	

The protein content of the alfalfa hay was sufficient in quantity and quality to establish a slight positive nitrogen balance. The nitrogen of the urine was not greatly increased over that in the period in which the low-nitrogen ration was fed, indicating a very good utilization (82 percent) of the absorbed nitrogen. From the energy metabolism data, the metabolizable energy of the alfalfa meal may be computed at 1,624 calories per kilogram of dry matter.

SUMMARY AND CONCLUSIONS

The digestion and metabolism of four pigs were studied while they were consuming (1) a ration containing 30 percent of oat hulls, with starch, sugar, and salts equal to 70 percent, and (2) a ration planned to contain paper pulp or cellophane to furnish as much crude fiber as the oat hulls in the former ration, together with starch, sugar, and salts. In the case of one pig, a third ration containing about 30 percent of alfalfa meal, and the rest of starch, sugar, and salts was studied.

The total digestible nutrients of the oat hulls as ordinarily computed amounted to 26.8 pounds per 100 pounds and those of the alfalfa meal to 31.3 pounds per 100 pounds. The metabolizable energy contents were 1,166 and 1,624 calories per kilogram of dry matter, respectively.

At the low levels of protein feeding prevailing, the absorbed protein of both feeds was almost completely utilized in metabolism.

The crude fiber of the oat hulls was digested to an average extent of 2 percent and that of the alfalfa meal to practically the same extent. The presumably pure cellulose of the paper, as well as of the cellophane, was digested to the extent of only 3.5 percent, on an average, the range for individual pigs being from 0 to 9.2 percent.

It appears from these studies that the crude fiber of oat hulls and of alfalfa meal, as well as pure cellulose, pass through the digestive tract of the pig almost, if not entirely, untouched by bacteria or other agencies of digestion. A possible explanation of the results of other experiments, apparently indicating a considerable digestibility of crude fiber by swine, is offered.

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AN APPARATUS FOR THE READY DETERMINATION OF AREAS OF COMPOUND LEAVES¹

By ROY E. MARSHALL

Research associate in horticulture, Michigan Agricultural Experiment Station

INTRODUCTION

A study made in 1932, involving extensive measurements of leaf areas in the black raspberry (*Rubus occidentalis* L.), required the development of a special technic. The blueprint and planimeter method had proved entirely too slow, and the matching method was not practicable because of the variability in shape of the leaflets. A method of direct translation, by reference to a table, of combined width plus length of leaflet into area was tested. This was based on a correlation ratio² of 0.972 between width plus length and area, found in 1,987 leaflets of fruiting canes, and a ratio of 0.994 found in 532 leaflets of current-season shoots. In practice this method required too much time. The leaf-punch method, though employed with great care and during most of the season in which the studies were made, gave values varying with different lots from 9 percent below to 19 percent above planimeter determinations; it was therefore not sufficiently accurate.

A correlation of 0.811 ± 0.095 was found between areas of 589 mid or end leaflets and the respective areas of compound or palmately trifoliate leaves, in fruiting canes. This indicated a close relationship between width plus length of end leaflets and area of compound leaves. Such corrected correlation ratios for 569 sample fruiting-cane leaves was 0.959 and for 407 leaves of current-season shoots it was also 0.959. These relationships indicated that a high degree of accuracy in area measurements of compound leaves would result from translating width plus length measurements of end leaflets into terms of leaf areas. To avoid tabular translation and to facilitate rapid and accurate measuring, the device here described was developed.

THE APPARATUS AND ITS UTILIZATION

The apparatus (fig. 1) consists essentially of two 50-cc hypodermic syringes (C) mounted at right angles to each other on the underside of a small table (D), the two syringes discharging into a common thick-walled rubber tube (E) (in later work a glass tube was used at E) connected with a vertical glass tube (A or B), which is mounted on a board calibrated in terms of areas of compound leaves. Attached to the pistons of the syringes are right-angle wires (F) which move across the field on the top of the small table (D) and on which the end leaflet is placed for measurement. When the pistons are so adjusted that the legs of the wires coincide with one side and the tip of an end leaflet, located at G, the area of the respective compound leaf is indicated on the scale at the top of the water column.

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² The curves for all correlation ratios in this paper are of the parabolic type, the relationships for the larger leaflets and leaves taking the form of a straight line.

The calibrated scale is such that when the pistons expel all the water from the syringes the top of the water column is at the top of the glass tube. This permits ready checking of the volume of water in the apparatus at frequent intervals.

The scale may be calibrated as follows: (1) Calculate the curve of best fit for width plus length of end leaflets and compound-leaf areas;

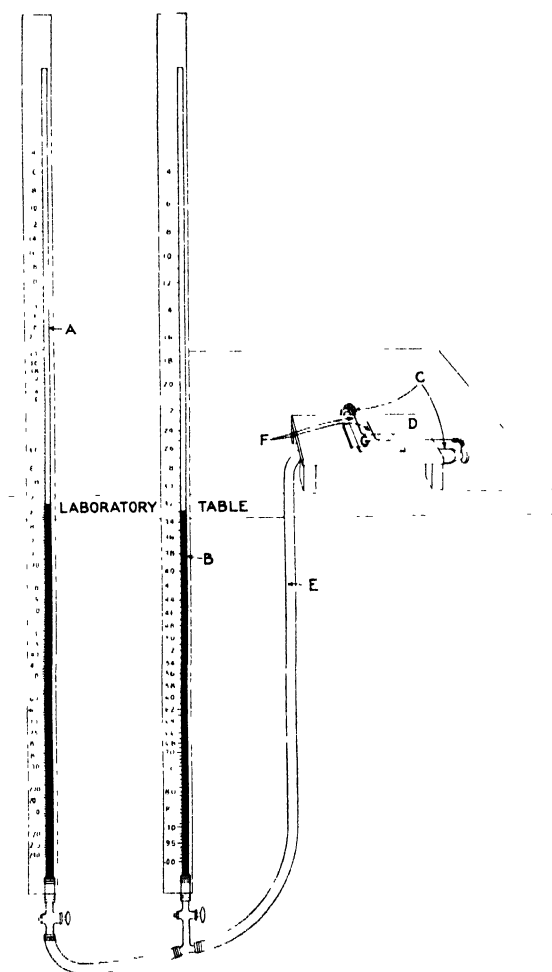


FIGURE 1 —Apparatus used in determining the areas of compound leaves of the black raspberry. The glass tube and calibration at A are designed to indicate the area of compound leaves of current-season shoots; those at B are designed to indicate the areas of the compound leaves of fruiting canes. See text for further description.

(2) alternately withdraw the plungers of the syringes one tenth inch at a time and record on the scale the corresponding compound-leaf areas indicated from the calculated curve; (3) interpolate; (4) the scale may then be further adjusted or corrected by using the apparatus to measure classes of end leaflets from compound leaves whose mean areas have previously been determined by the planimeter.

Inasmuch as the same relationships between width plus length of end leaflets and compound-leaf areas do not exist in leaves of fruiting canes and current-season shoots of the black raspberry, a scale for each must be calibrated. Furthermore, maximum individual leaf areas of current-season shoots are more than twice as great as those from fruiting canes.

Though the standard deviation between planimeter measurements and those obtained by the use of the apparatus amounted to 11 percent of the mean compound-leaf area for leaves of fruiting canes and 12.9 percent for leaves of current-season shoots, the total error³ for 898 fruiting-cane leaves (a random sample from leaves collected between June 20 and July 29) was +3.7 square inches, or 0.15 percent, and the error for 373 leaves of current-season shoots was -11.2 square inches, or 0.55 percent. In measuring leaves from a single cane or shoot the error involved would probably be greater because of the smaller number involved. However, the method appears to approximate closely the accuracy of the planimeter, is much more rapid, and makes possible the handling of a much larger number of leaves, thus permitting more representative sampling.

³ Total error refers to the total difference in areas as indicated by the planimeter and apparatus methods. For leaves of fruiting canes the indicated areas were 2,526. and 2,529.7 square inches, respectively, and for leaves of current-season shoots, 2,034.3 and 2,023.1 square inches, respectively.

THE RECOVERY OF SALMONELLA PULLORUM FROM THE FECES OF ADULT CARRIERS OF PULLORUM DISEASE¹

By R. E. GREAVES, *associate in poultry science*; R. S. DEARSTYNE, *head, Department of Poultry Science*; and H. C. GAUGER, *instructor in poultry science, North Carolina Agricultural Experiment Station*

INTRODUCTION

The results of recent studies of pullorum disease of fowl indicate that adult bird carriers of this disease when confined with adult negative birds are a menace to the well-being of the negative birds. A slow progressive transmission of the infection in such cases has been demonstrated (1, 2, 4, 5, 6, 9).² Rettger and Plastringe (8), p. 178, after reviewing the work on this subject, state: * * * "It is safe to assume that *Bacterium pullorum* is readily transmitted from one adult bird to another through infected droppings." This paper offers additional proof that the causative organism of the disease can be recovered from the droppings of the adult carrier bird, and that such droppings may be a source of infection for adult negatives.

The importance of this problem is emphasized by the fact that in a number of States only a single annual test is applied to breeding flocks, and the detection and removal of reactors in this test are accepted as showing that a clean flock remains. The fallacy of this procedure was noted by Dearstyne, Greaves, and Gauger (4) in a report of the results of tests made by the North Carolina State Department of Agriculture in 1930. An initial test on 37,893 birds showed 3,194, or 8.43 percent, to be positive. Subsequent tests of those showing a negative reaction in the first test revealed 1,859 more reactors, bringing the total to 5,053, or 13.33 percent of the total number studied. These figures indicate that 36.8 percent of the total reactors remained in the flocks undetected by the first test. Obviously these birds were subjecting the adult negative birds to the chance of contracting the disease through contamination resulting from cohabitation. The results of experimental work with three flocks of reactors owned by the North Carolina Agricultural Experiment Station, showed that of 27 reactors, 12, or 44 percent, were intermittent to the test; of 26 reactors, 18, or 69.2 percent, were intermittent; and of 26 reactors, 19, or 73.1 percent, were intermittent.

The purpose of the investigation here described was to determine whether the carrier birds void *Salmonella pullorum* in their droppings, and if so, whether the recovered organisms are sufficiently virulent to produce the disease in negative adults and chicks when administered per os.

METHOD OF STUDY

The subjects used in this work were proved adult carriers that had been subjected to a semimonthly serological test for 2 years or more. The birds were brought from range and placed in individual

¹ Received for publication Jan. 3, 1933; issued October, 1933.

² Reference is made by number (italic) to Literature Cited, p. 446.

coops with wire bottoms, under which layers of clean paper were placed to catch the droppings.

During a period of 2 months 281 specimens of feces from 16 carrier birds were collected. These were placed in 4-ounce bottles containing 35 to 40 cc of meat-extract broth having a reaction of pH 6.6, and were incubated for 24 hours at 37° C. Approximately 1 cc of this broth was then inserted into sterile Petri dishes, and this was flooded with 2 percent base agar. The broth and agar were enriched with brilliant green in the proportion of 1:150,000. When the agar had solidified, the plates were inverted and incubated for 24 hours at 37° C. Various organisms, as judged by distinct colonies, were inserted into lactose fermentation tubes to which Andrade's indicator had been added. The results of these tests were negative for *Salmonella pullorum*.

Some difficulty in securing pure cultures was experienced in the preliminary tests, but this was overcome by improvement in technic. Two percent peptone water, as advocated by Rakieten and Rettger (7), was prepared. The hydrogen-ion concentration of this medium was so adjusted that it varied from pH 6.6 to 6.8 and was buffered with 1 percent of a 10-percent solution of potassium phosphate (KH_2PO_4 and K_2HPO_4) having a pH range of 6.6 to 6.8. Brilliant green was added to this medium in the proportions of 1:200,000, this dilution having been shown to inhibit the growth of colon organisms in cleared broth without causing serious suppression of the growth of *Salmonella pullorum* and *S. gallinarum*. Under aseptic conditions 5-cc amounts of this medium were placed in small test tubes. In these tubes fecal specimens one half to one fourth the size of a pea were planted. The inoculated broth was shaken thoroughly, placed in the incubator, and kept there for 6 to 8 hours at 37° C. After incubation, one half to 3 cc of the top fluid was poured into bottles containing 35 to 40 cc of cleared meat-extract broth to which brilliant green had been added, the amount used in each case depending on the turbidity present. The cultures were then incubated for 18 to 24 hours at 37° and streak tests made from them on brilliant-green agar plates. The streaked plates were inverted and incubated for 24 hours at 37°, and colonies from these cultures were inoculated into lactose fermentation tubes or into indicator plates prepared by the addition of 1 percent lactose and 1 percent Andrade's indicator to base agar. Three fecal samples were taken daily from the birds kept for study. Control cultures, seeded with *S. pullorum* and a fecal specimen from one of the carrier birds were used each day. In all, 236 fecal samples from 21 birds were examined.

RECOVERY OF THE ORGANISM

In this study *Salmonella pullorum* was recovered from the feces of two birds, designated by leg bands 1631 and 1862. *S. pullorum* was recovered from only 1 of 3 fecal samples taken on the same day from bird 1631; it was not recovered from 3 fecal samples taken on each of the sixth, seventh, tenth, and twelfth days subsequent to, nor on the day prior to, the recovery noted. *S. pullorum* was obtained from all of 3 fecal specimens taken from bird 1862 on the first day, but was not recovered from 3 fecal specimens taken on the first, fourth, and sixth days subsequent to the recovery of the organism.

The organisms were determined as *Salmonella pullorum* by the morphological, cultural, and biochemical properties of the strain as noted in the Report of the Conference of Official Research Workers in Animal Diseases (3).

The two birds from which the recoveries were made were Barred Plymouth Rocks and had the following histories:

Bird 1862 was hatched April 28, 1928. Its sire and dam were both reactors to tests for pullorum disease. The first positive test on this bird was secured 63 days after it was hatched. The bird reacted strongly to the test in all except 1 of 76 tests made at semimonthly intervals, the single negative test being secured when the bird was 13 weeks old. The serum titer ran 1:1,000 or higher in 26 of the 76 tests. There was a marked increase in positive trend of the serum titer as the bird matured. This bird was an intensive producer, having laid 519 eggs prior to the time of fecal examination. One hundred of these were positive for *Salmonella pullorum*. The 4 eggs laid by this bird immediately before the isolation of *S. pullorum* from the feces were positive for the organism.

Bird 1631 was hatched April 29, 1929. Its sire and dam were both reactors to tests for pullorum disease. The first positive test on this bird was secured 73 days after it was hatched. The bird was a reactor of the intermittent type, 36 of 64 agglutination tests made at semimonthly intervals being positive. The serum in all tests showed a titer that did not exceed a dilution of 1:50. Of 306 eggs laid and tested bacteriologically prior to the recovery of *Salmonella pullorum* from the feces, only 7 were positive for the organism. The serum of the bird was positive for *S. pullorum* agglutinins in a dilution of 1:25 in 4 tests made at 2-week intervals prior to the recovery of the organism from her feces.

INOCULATION TESTS

The three recoveries from bird 1862 were administered to three Single-Comb Rhode Island Red pullets that had proved negative for pullorum disease in three successive tests at 30-day intervals. The birds were inoculated per os with a 24-hour bacillary saline emulsion of the strains recovered from the fecal material. Serum was secured from these birds at intervals as noted subsequent to these inoculations and titrated for agglutinins with a stock pullorum antigen. The dosages given and the results obtained are noted in table 1.

TABLE 1.—*Serological tests of three Single-Comb Rhode Island Red pullets after inoculation with Salmonella pullorum secured from bird no. 1862*

Pullet no.	Dosage	Serological tests indicated periods after inoculation		
		11 days *	18 days	48 days
1326	Cc 1	Positive 1:50	Positive 1:2,000	Positive 1:1,000
1388	2	Positive 1:50	Positive 1:300	Positive 1:200
1315	3	Positive 1:50	Positive 1:2,000	Positive 1:1,000

* Dilutions run 11 days after inoculation were in 1:25 and 1:50 strength only.

Subsequent to inoculation these birds laid 43 eggs, and *Salmonella pullorum* was recovered from 13 of these—2 from bird 1315, 6 from bird 1326, and 5 from bird 1388. Inoculation studies with these strains were likewise made with six Rhode Island Red chicks at 3 days of age. These were given graduated doses of the same strains used in the inoculation studies of adults. One chick died of bacteremic pullorum disease. Fifty days after inoculation 3 of the 5 surviving chicks showed agglutinins against a pullorum-disease antigen in a 1 : 25 dilution.

The strain isolated from bird 1631 was inoculated into 2 Single-Comb Rhode Island Red pullets and 3 hens of the same breed. These birds had given negative reactions for pullorum disease in three successive tests conducted at intervals of 30 days. They received inoculations per os of a 24-hour bacillary saline emulsion of the strain isolated from bird 1631. Serum was secured from these birds at intervals as noted subsequent to these inoculations and titrated for agglutinins with a stock pullorum antigen. The dosages given and results secured are shown in table 2.

TABLE 2.—*Serological tests of 2 Single-Comb Rhode Island Red pullets and 3 hens of the same breed after inoculation with Salmonella pullorum secured from bird 1631*

Bird no.	Dosage	Serological tests indicated periods after inoculation		
		8 days	14 days	140 days
718	Cc 3	Positive 1:25	Doubtful 1:25	Negative.
1106	3	Negative	Negative	Do.
1143	1	do.	do.	Do.
1392	3	do.	do.	Doubtful 1:25.
1361	2	do.	Positive 1:25	Positive 1:25.

Subsequent to inoculation the birds laid 34 eggs, and *Salmonella pullorum* was obtained from 1 egg laid by bird 1361. Chick inoculations from the strains used failed to produce demonstrable agglutinins against an antigen of *S. pullorum*.

OBSERVATIONS ON THE PRESENCE OF THE BACTERIOPHAGE

During the course of this experiment four controls gave negative results, but a fifth yielded several small colonies, which were transferred to indicator plates. After incubating for 24 hours these colonies presented a moth-eaten appearance. After 48 hours they were almost completely lysed and could not be transferred. This indicated that a bacteriophage was active in some of the fecal dilutions, and negative cultures from all birds were accordingly cross-streaked with a broth culture of *Salmonella pullorum*, lysis being evident where the new and old streaks crossed when the phagic principle was sufficiently strong. In this manner the lytic agent was detected on one or more plates from 5 of the last 13 birds studied.

SUMMARY

An organism with the true morphological, cultural, and biochemical characteristics of *Salmonella pullorum* was isolated from the feces of 2 of 21 known adult carriers of pullorum disease.

The isolations recovered were obtained from one bird of proven constant reaction to the test for pullorum disease, and from one bird of proven intermittent reaction.

The organisms isolated from the feces of these birds produced the disease when administered per os to proven negative adult birds, and one strain produced the disease in 3-day-old chicks when administered in the same way.

Lytic factors that were sufficiently strong to prevent the recovery of *Salmonella pullorum* from the fecal samples were repeatedly encountered.

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SOME FACTORS INFLUENCING THE VARIABILITY IN LENGTH OF COTTON FIBERS ON INDIVIDUAL PLANTS AS SHOWN BY THE SORTER METHOD¹

By G. M. ARMSTRONG, *botanist and plant pathologist*, and C. C. BENNETT, *research assistant, South Carolina Agricultural Experiment Station*²

INTRODUCTION

It is a well-known fact that individual fibers on a seed of cotton differ in lengths. The length of staple as determined by the ordinary method of pulling and combing refers to only one or two of the largest groups of fibers of approximately the same length, and gives no information as to the relative proportions of short fibers or very long fibers. By the use of the Baer and Johannsen mechanical fiber sorters, fibers were separated into groups according to length. Variations between samples were thus revealed. Such methods when used on samples taken from bales of cotton in commerce may yield little information of value to the plant physiologist or plant breeder, but when used on samples obtained from the seed of a variety grown under known conditions, they show some significant differences. A study of such material was undertaken to determine some of the factors which affect lint length.

EXPERIMENTAL METHODS

In 1929, seed of *Gossypium hirsutum* L., variety Super-Seven, strain 4, was obtained directly from the breeder and planted in both greenhouse and field. The field plot, of Cecil clay loam, which usually yields about a bale per acre if given liberal applications of phosphorus, potash, and nitrogen, was divided so that one half received these materials and the other half received none. In the greenhouse, plants were grown directly in the floor soil. They were given liberal applications of commercial fertilizer and watered regularly. The plants were normal, but attained a considerably greater size than those grown in the field. A distinct second crop of mature bolls was produced in the greenhouse but not in the field.

Small groups of plants were selected from various sections of the plots, uniformity in size and spacing being particularly noted. All bolls on the selected plants were self-pollinated. Each boll was tagged on the day of blooming, the branch number and position on the branch being recorded on the tag. The open bolls were picked regularly, usually twice a week, and the date of picking noted.

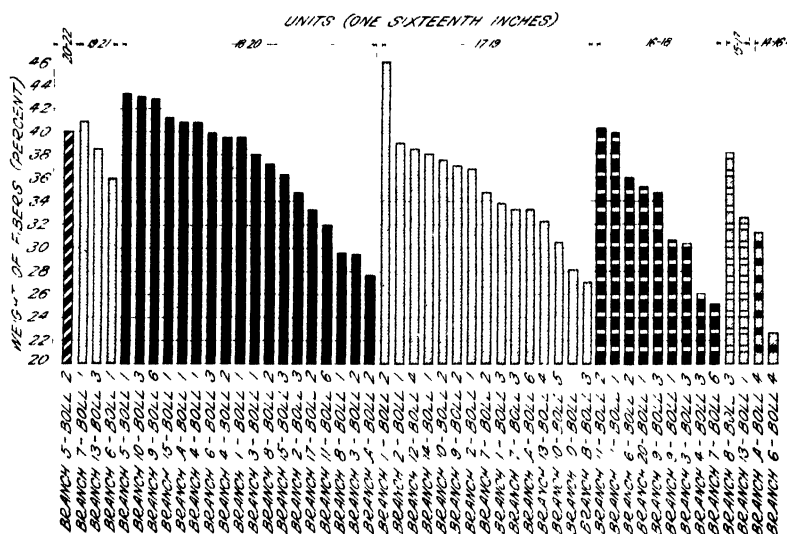
Seed produced from selfed flowers of 1929 was available for planting in 1930, and all flowers on selected plants were again self-pollinated as during the preceding season. In the field and the greenhouse the same plots were used as in 1929, and the general procedure was the same.

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² The authors are indebted to Director H. W. Barre, coleader of the project, for the helpful suggestions given during the progress of this work.

A suitable room for arraying the fibers was available through the courtesy of the textile department of Clemson College. The humidity of the room was kept at 70 percent by automatically controlled humidifiers. The normal variation was within a range of 1 percent. Both Johannsen and Baer sorters were used by the junior author, who made the arrays. The fibers were weighed on a Zweigle balance which was checked against two analytical balances of precision. To insure a representative sample for the sorter, the fibers were carefully pulled by hand from every seed in each boll and thoroughly mixed in the manner described by Balls (5, p. 59).³

The methods of measuring the daily growth of fibers, the volume of seed, the volume of boll, and the number of fibers per seed are described later in connection with the discussion of these operations.



by numbers. For the sake of brevity certain symbols are used in the discussion which follows; for example, 6-4 indicates the boll at the fourth node on fruiting branch no. 6. To translate the sorter distribution into the approximate terms of the cotton classer, the modal length has been employed. The percentages of the total weight of the three heaviest groups which are adjacent to each other have been added. For example, the boll 6-4 had 7.14 percent of the total lint weight fourteen sixteenths of an inch in length, 8.27 percent fifteen sixteenths of an inch, and 7.22 percent 1 inch. This gives 22.63 percent, as shown in figure 1. The cotton classer would probably call this a cotton with fibers fifteen sixteenths of an inch long, since the lint length of the classer is usually about the 75 percent point on the cumulative weights scale of the sorter array. The term "modal groups", used for the presentation of certain features of the length characteristics of the fibers on a plant, does not adequately describe their chief characteristics, but neither does the mean nor the practical length obtained by the hand-stapling method. Clegg (11) shows very clearly that no one expression of results is entirely satisfactory and that a combined stapling test is necessary to bring out such important features of a cotton sample as practical length, percentage dispersion as a measure of uniformity, and percentage of short fibers. A combination of the three heaviest length groups does not give a real measure of the uniformity of fiber length but gives some indications of it. A majority of such modal groups are also the same as the practical length of the hand-stapling test.

In figure 1 the fiber of boll no. 4 on branch no. 6 is the least desirable from the standpoint of length and uniformity. Perhaps the greatest contrast to boll 6-4 in modal groups is 1-2, with 46 percent of the lint in the three heaviest adjacent groups. The fibers of this boll would probably be classed as eighteen or nineteen sixteenths of an inch in length. The boll containing the longest fibers is 5-2, the fibers of which would be classed as twenty-one sixteenths of an inch. The relation of boll position to fiber length and uniformity is discussed in succeeding paragraphs, but it is desired to point out here the six sixteenths of an inch difference in fiber length of two bolls 6-4 and 5-2 located rather close together on the plant. This is a greater difference in fiber length than that usually supposed to exist between varieties bred for variations in length of staple.

A variation of five sixteenths of an inch in fiber length from boll to boll was observed in several of the plants grown in the field, though none of those selected for study produced more than 10 mature bolls.

Humbert and Mogford (18) used the combing method to study the lint length of every seed in each lock of an inbred plant of Mebane cotton and also of a few seeds from its progeny. They found that the length of lint varied in different bolls on the same plant, in the same boll, and on seed which were side by side in the same lock. The results here presented show the same essential variation in regard to different bolls. The lint produced in bolls near the top of the Mebane plant had a mean length that did not differ greatly from the length of lint in bolls from other parts of the plant. The results presented in figure 1, for a plant grown in the greenhouse, are in essential agreement with this finding, since boll 13-3 ranks third, 10-3 ranks sixth, and 15-1 ranks eighth. Bolls at the top of plants growing in the field, however, showed a tendency to produce shorter lint. Ewing (19)

and Venkataramanan (25) also found shortening of lint in upper bolls, probably due to increasing drought, but Kearney and Harrison (20) found that bolls borne on the lower fruiting branches of the Pima variety of Egyptian cotton produced shorter fibers than bolls higher on the plant.

Not only were there decided differences in the length of the modal groups, but there were also appreciable differences in the uniformity of distribution of the fibers of various lengths, as is seen in figure 2. The curve for boll 1-2 shows a greater tendency to flatten, and the boll therefore possesses a rather large percentage of fibers in a few length groups, 46 percent falling in the groups seventeen to nineteen sixteenths of an inch. Boll 6-4 has only 22.63 percent of its fibers in any three adjoining length groups, whereas boll 5-2 is intermediate in this respect with 40 percent of the fibers in three groups. If a

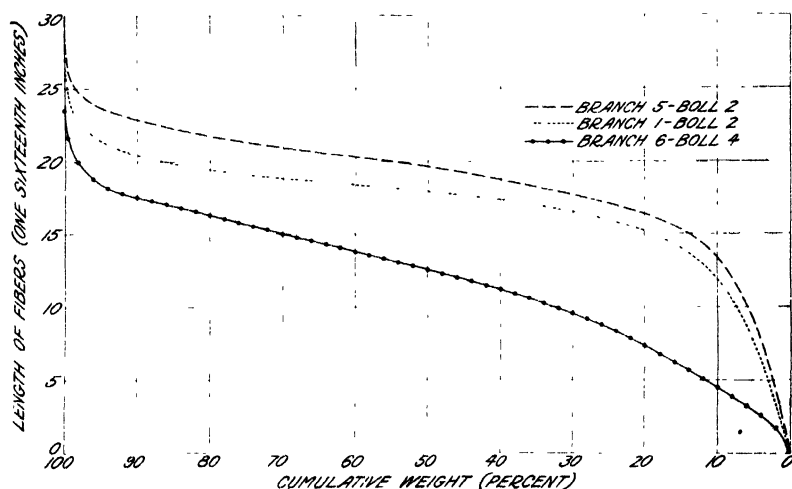


FIGURE 2 - Variation in the distribution of the length groups of sorter arrays of the lint from three bolls of greenhouse plant no. 13.

uniform staple of a particular length is really desired, then boll 1-2 is perhaps the best on this plant, though there are on the plant 14 other bolls that the cotton classer would probably grade as the same length and 22 others that have longer staple lengths.

A study of lint length finally resolves itself into a study of cell nutrition, which is complicated by numerous variable factors. Perhaps the single seed would be the best unit for study of the competition between seed and lint for the available nutrients, but for practical reasons the boll has been used as the unit in this study. The following points were considered in their relation to lint length on individual plants: The effect of (1) position of seed in a single boll, (2) position of bolls of the same age on a single plant, and (3) age as well as position of bolls on a single plant.

EFFECT OF POSITION OF SEED IN A SINGLE BOLL

Only a few arrays were made of the fibers of every seed in a single lock. As an example of the variation to be found, the results from a lock of boll no. 1 on the sixth fruiting branch of plant no. 6, grown in

the greenhouse, are given in table 1. If the percentage weights of the three heaviest adjoining length groups are used as the probable staple length, it will be seen that seeds nos. 1 and 2 at the base of the lock have the shortest lint length, which is seventeen sixteenths of an inch. Seeds 3, 4, and 8 have fibers nineteen sixteenths of an inch in length; seeds 5, 7, 9, and 10, twenty sixteenths of an inch; and seed 6, twenty-one sixteenths of an inch. Thus, there is a variation of five sixteenths of an inch in lint length of the seed from a single lock.

TABLE 1.—Sorter distribution of fiber lengths on each seed from a l

[Seed numbered from the base of locule upward]

Lengths in sixteenths of an inch	Percentage of the total fiber weight in each length group for seed no.—									
	1	2	3	4	5	6	7	8	9	10
29							0.02			
28									0.02	
27			0.09			0.14	.09	0.04		
26	0.09		1.35	0.10	0.10	.23	.19	.09	.17	0.06
25	.09	0.16	1.13	.86	.77	1.05	1.49	.36	.27	.20
24	1.50	.24	2.78	1.44	2.08	3.67	3.45	1.26	1.65	.80
23	5.02	.47	4.70	4.12	5.04	6.38	6.16	2.07	4.55	4.82
22	5.28	1.98	4.00	5.94	8.43	11.71	8.96	4.68	8.27	9.23
21	8.80	3.88	8.96	9.38	9.29	12.15	17.64	10.08	15.51	14.65
20	10.48	6.33	12.96	12.83	13.70	16.69	13.85	17.28	15.51	13.85
19	12.15	10.93	14.61	12.83	13.70	8.91	13.63	22.94	12.41	10.64
18	12.41	13.85	10.26	10.44	6.90	6.38	12.14	11.52	11.99	10.54
17	13.82	14.73	10.61	8.10	7.85	4.54	5.60	6.48	8.89	9.23
16	13.12	12.51	6.09	5.75	5.66	4.28	4.48	5.22	3.72	6.42
15	4.75	7.76	4.52	6.90	4.98	4.37	2.05	2.79	2.70	3.81
14	3.26	6.02	3.13	2.87	3.54	4.20	1.31	1.98	3.10	2.61
13	1.94	5.86	1.91	1.92	3.06	1.57	1.12	2.43	1.65	2.01
12	.62	3.40	1.91	1.21	1.72	1.75	.56	1.44	.83	1.10
11	1.06	2.06	1.57	1.44	.57	1.57	.19	1.17	.83	.50
10	.79	1.82	1.39	1.44	1.19	1.31	.28	.81	.83	.50
9	.79	.95	1.22	1.05	.96	.87	.56	1.08	.72	.90
8	.53	1.11	.87	1.72	1.72	.87	.75	.72	.52	.50
7	.26	.95	.70	1.24	1.34	1.22	.56	.36	.52	.80
6	.44	.63	1.04	1.72	1.15	1.40	1.08	.72	.89	.50
5	.44	.55	.87	1.15	1.15	1.05	.75	.81	.41	1.20
4	.44	.95	1.22	1.53	1.44	1.14	.65	1.08	1.24	1.20
3	.97	1.74	.87	1.53	1.34	.96	.93	.81	1.03	1.40
2	.97	1.11	2.26	2.49	1.82	1.57	1.49	1.80	1.65	2.51

* Boldface figures show modal length and emphasize increase in length of lint from seed 1 to 6 and then a slight decline.

If a curve were drawn through the boldface lint lengths in table 1, it would serve to emphasize the increase in length of lint from seed 1 to seed 6, inclusive, and then the slight decline in seeds 7, 8, 9, and 10. Henry (17) notes the same general trend of lint length from base to tip of a lock in Egyptian and sea-island cottons. Humbert and Mogford (18) call attention to the variation in length of lint from seeds located side by side in a lock. Ayvar and Rao (1) state that in a lock of *Gossypium hirsutum* grown in India the first seed produced the shorter lint, and there was no difference in length of lint among the other seeds in a lock.

The distribution of the various lengths is also quite variable, as table 1 shows. If we assume that a sample desired for spinning is one with the greatest percentage of the weight in closely adjoining length groups, and arbitrarily take those groups with 10 percent or more in each for comparison, the fiber from seed 1, with 61.98 percent of the weight in 5 length groups, is the best, and that from seed 5, with 27.4 percent in 2 length groups, is the poorest. Seed 8, with 61.82 percent of the fibers in 4 length groups, is a close second to seed 1, according to the assumed standard of uniformity.

A comparison of the seeds on the basis of the percentage of fibers less than eleven sixteenths of an inch in length also places seed 1 first, with 5.63 percent of the fibers in this group, while seed 4, with 13.87 percent in this group, would probably have the greatest waste of short fibers in the spinning process. Seed 6, with the longest fibers, has 10.39 percent of the above-mentioned short fibers.

EFFECT OF POSITION OF BOLLS OF THE SAME AGE ON A SINGLE PLANT

Because of the impossibility of obtaining many bolls from flowers of 1 day on a single plant, a comparison was made of several bolls from flowers on 2 successive days, namely, July 8 and 9 and July 21 and 22. Bolls 2-3, 3-3, 5-2, 6-2, and 8-1 developed from blooms on the first-mentioned dates, and curves of the arrays are shown in figure 3. There is a difference of four sixteenths of an inch in length of lint of modal groups between bolls 3-3 and 5-2 and also a very appreciable

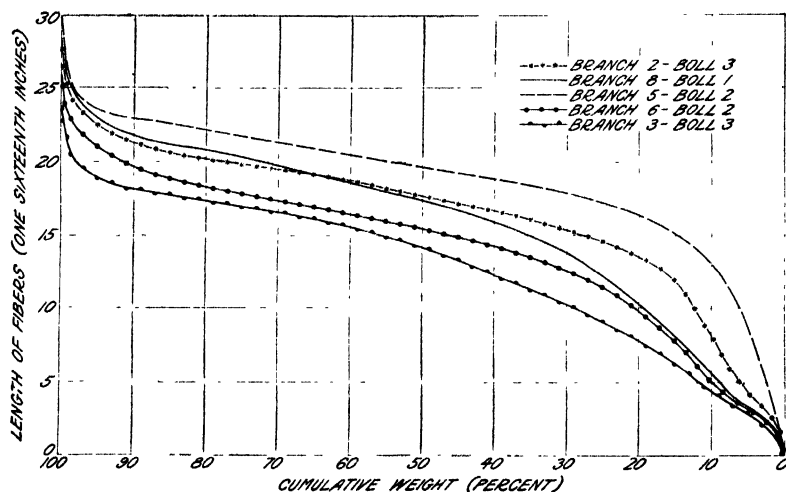


FIGURE 3.—Sorter arrays of lint from bolls flowering July 8 and 9 on plant no. 13.

difference in the distribution of the different lint lengths, boll 3-3 being inferior to boll 5-2.

Four bolls, 8-3, 9-3, 11-2, and 13-1, developed from blooms on July 22, and one, 6-4, from a bloom on July 21. In the first-mentioned bolls there was considerable uniformity in the distribution of the various lint lengths (fig. 4), but in boll 6-4 there were decidedly more short fibers. These examples, as well as the results from other plants, which will not be presented, show that even though the bolls may develop under about the same external environmental conditions, there is much variation in the distribution of the fibers of various lengths and in the final length attained.

EFFECT OF AGE AS WELL AS POSITION OF THE BOLLS ON A SINGLE PLANT

POSITION ALONG THE MAIN AXIS

The curves from the arrays of fibers for all bolls that matured on the first node of each fruiting branch from nos. 1 to 20, inclusive, are shown in figure 5. Bolls 8-1 and 10-1 are outstanding because of the

percentage of short fibers that they contain, although in figure 1 they are classed as nineteen sixteenths and eighteen sixteenths of an inch in length, respectively. It appears from table 2 that conditions for lengthening of lint in bolls at the first node were favorable for those on branches 1 to 7, inclusive, the longest lint (twenty sixteenths of an inch) occurring in bolls 6-1 and 7-1. Except for the slight variation in bolls 9-1 and 11-1, conditions then appeared to become progressively less favorable, since lint length for boll 8-1 was nineteen sixteenths of an inch, for 10-1 and 12-1 eighteen sixteenths of an inch, and for 13-1 only 1 inch. Conditions apparently again became more favorable, as lint length was eighteen and nineteen sixteenths of an inch for bolls 14-1 and 15-1. Unfavorable conditions for bolls at the first node followed, as no bolls were retained at first nodes on branches 16, 17, 18, and 19, though a boll at the first node on branch 20 produced relatively short lint, seventeen sixteenths of an inch in length. Even

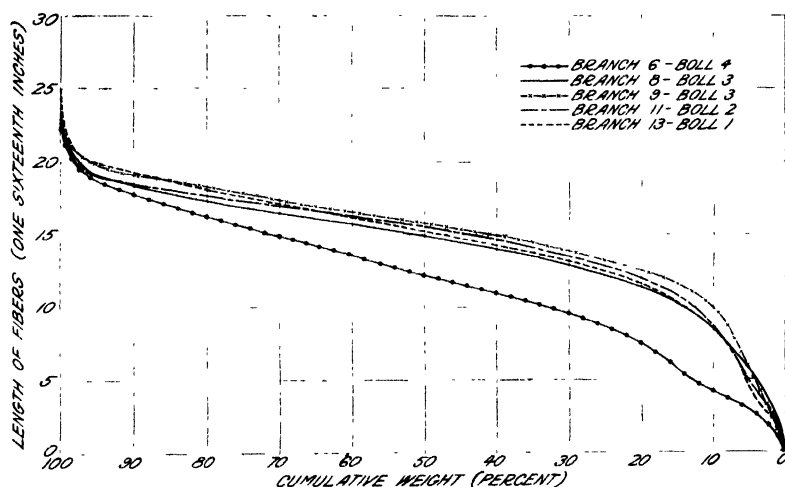


FIGURE 4 — Sorter arrays of lint from bolls flowering July 21 and 22 on plant no. 13

though it has been previously shown that there are differences in lint characteristics of bolls from flowers of the same day or for periods of 2 or 3 days, indicating that specific nutritional conditions may be localized to some extent, such progressive changes as the foregoing seem to indicate that gradual changes in those factors that influence the nutrition of seed and lint are also operative. Two factors of indicated importance are water supply (3, 4, 21, 23) and boll load (12), though much remains to be elucidated in this regard.

In spite of the very decided variation from boll to boll, evidence of some generalized effect is also strengthened if other bolls than those of first nodes are considered. It will be seen in table 2 that bolls from blooms of June 19 to July 8, inclusive, produced relatively long fibers in the modal groups, with a tendency for longest fibers to be produced in bolls from flowers that opened near the latter date. Quite unfavorable conditions apparently occurred from bolls beginning development July 9, 10, and 11, since lint length was two to four sixteenths of an inch shorter than in bolls beginning development July 8 on comparable positions of adjacent limbs.

TABLE 2.—Date of blooming, position of boll on the plant, and modal lint length for each cotton boll on fruiting branches of plant no. 13, 1929

Date of blooming	Bolls produced on—				Modal lint length ^a ^b in sixteenths of an inch			
	First nodes	Second nodes	Third nodes	Fourth, fifth, and sixth nodes	First nodes	Second nodes	Third nodes	Fourth, fifth, and sixth nodes
June 19	1-1				19			
June 23	2-1				(19) 18			
June 26	3-1				19			
June 29		1-2				(19) 18		
June 30	4-1				19			
July 2	5-1				(20) 19			
July 3		3-2				19		
July 4	6-1				20			
July 5		4-2				(20) 19		18
July 6	7-1		1-3		20			
July 8		5-2	2-3			21		19
July 9	8-1	6-2	3-3		19	17	(16) 17	
July 10	9-1				(18) 17			17
July 11			4-3					
July 12		7-2				(19) 18		
July 14	10-1				18			
July 15		8-2	6-3			19	(20) 19	
July 16	11-1	9-2			(19) 17	18		
July 17			7-3				18	
July 18	12-1				18			
July 20		10-2				18		
July 21				6-4				15
July 22			8-3				16	
July 22	13-1	11-2	9-3		(17) 16	17	17	
July 24	14-1				18			
July 27			10-3				(20) 19	
July 28	15-1				19			
Aug 3			13-3				20	
Aug 4								
Aug 5				7-6				19
Aug 5				12-4				18
Aug 8		17-2		13-4		19		(19) 18
Aug 9			15-3	10-5			19	18
Aug 9				9-6				19
Aug. 10.	20-1				(18) 17			

^a The cotton classer's staple length is usually about the 75 percent point on the cumulative weights scale of the sorter array. The figures in parentheses give the length nearest the 75 percent point when these do not coincide with the modal grouping.

^b Boldface figures show shortest fibers which were produced from blooms at 2 short intervals of time.

Lint of greater length was produced in all bolls from succeeding blooms until those appearing July 21 and 22, which produced the shortest lint of all, namely fifteen, sixteen, and seventeen sixteenths of an inch. Later bolls produced longer lint except 20-1, the last one of the first crop to be retained, which produced lint of seventeen sixteenths of an inch. These seem to be rather striking variations in lint lengths in bolls from flowers of successive days. Balls (4) also mentioned, " * * * the extreme rapidity with which the properties of the cotton may change, so that the picking of 1 day may be capable of differentiation from those of the preceding and following days."

POSITION OF BOLL ON THE BRANCH

In addition to the position of the boll along the main axis, which has been considered, its position on the branch may also influence lint length and uniformity.

An examination of table 2 shows that fruiting branches 1, 3, 4, 6, 7, 8, 9, and 10 produced bolls at the first three nodes and branch 6 also produced a boll at the fourth node. There are variations of three

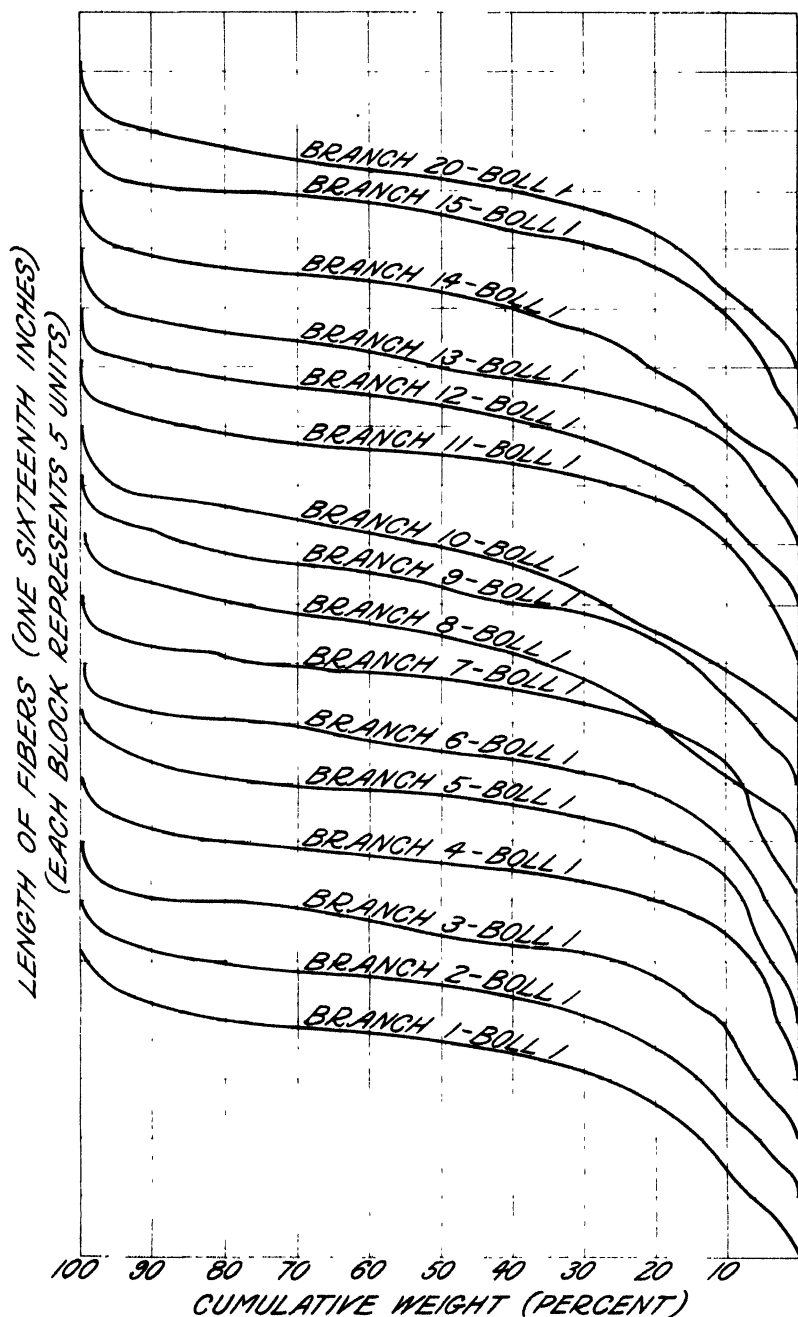


FIGURE 5.—Sorter arrays of lint from all bolls maturing at the first node on each fruiting branch of plan no. 13. Each block represents 5 units.

sixteenths of an inch in the lint length of modal groups from bolls at 3 nodes on 1 limb and of five sixteenths of an inch between the 4 bolls on branch no. 6. The inferiority of boll 6-4 should perhaps be given further consideration. Branch 6 is in an intermediate position along the main axis, which may be considered a fairly favorable position for the transfer of water and organic materials, though the boll at the fourth node may be considered in a relatively unfavorable position because of the three bolls ahead of it on the branch. Three other fruiting branches, however, nos. 7, 9, and 10, produced four bolls on each branch. If the bolls are given a rank according to their position in figure 1, from left to right, the fourth retained boll on each of these branches—namely, 7-6, 9-6, and 10-5—ranks 46, 6, and 35, respectively. This would indicate that the position along the branch is not necessarily a major factor in the inferiority of boll 6-4.

Boll 6-4 developed from a bloom of July 21, and it has already been shown that all bolls developing July 21 and 22 produced relatively short lint, regardless of their position on the plant. Some factor or factors acting throughout the plant on bolls from flowers of these dates as well as on those produced June 9, 10, and 11 apparently had a greater effect on the shortening of lint than any of the more specific and localized influences that are considered in this paper.

The lint from all the bolls from five plants in the greenhouse was arrayed. Two other plants, 19 and 20, in addition to 13, show the alternating production of longer and shorter lint, but not so distinctly as does the latter. Such a condition is not evident in the two remaining plants of the five which were studied. Bolls from distinctly different flowering dates on each of the greenhouse plants 13, 19, and 20 show the shorter lint, indicating that it was not general environmental conditions in the greenhouse that produced this condition.

Studies of bolls from the field also show no specific relation of lint characteristics to position on the branch. Fifty limbs from field plants with three or more bolls per limb were selected for study in 1929, and arrays of the fiber from all bolls on 14 of them were made. Fruiting branches 4, 5, and 6 were selected chiefly in order that bolls of comparable ages might be had. Differences in lint length of modal groups or the percentage of fiber ten sixteenths of an inch or less from boll 1 to boll 2 or boll 2 to boll 3 are not significant, though there is a tendency for the fibers to be longer in bolls at the second and third nodes. The figures for the percentage weight of the modal groups seventeen, eighteen, nineteen, and twenty sixteenths of an inch are as follows: For bolls at the first node, 12.79, 14.26, 12.72, and 9.51; for bolls at the second node, 11.25, 12.58, 13.67, and 10.38; for bolls at the third node, 10.76, 11.89, 13.03, and 11.09. The percentage of fibers ten sixteenths of an inch or less at node 1 is 9.47; at node 2, 9.67; and at node 3, 10.39.

PROBABLE TIME OF ORIGIN OF FIBERS AS RELATED TO VARIATIONS IN LINT LENGTH

The majority of the sorter graphs made of lint from the variety of cotton used in these studies approximate the one shown in figure 8 for fibers from 50-day-old bolls. A knowledge of some of the factors which produce this usual type of distribution is necessary before the variations from the type can be at least partly understood.

Balls (3) and Hawkins and Serviss (16) have stated that the cotton fibers elongate during the first 25 days of boll development, and that wall thickening takes place during the following 25 days. Balls (3, 4) maintains that the individual cells of the epidermis of the seed all sprout at the same stage of boll development, on or near the day when the flower opens. If this is accepted as true, then a

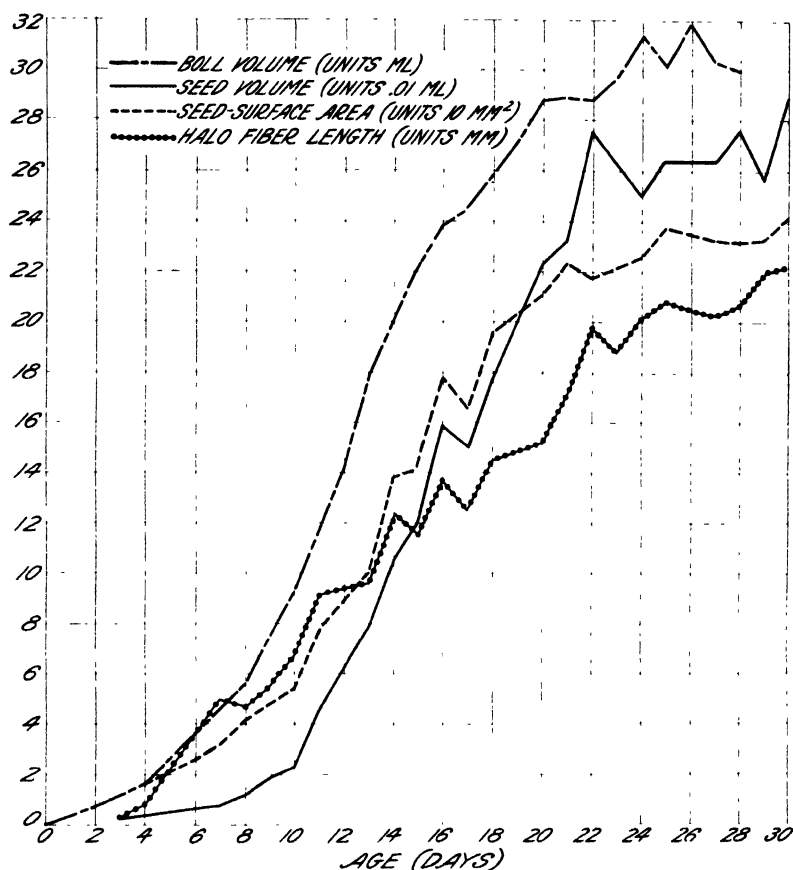


FIGURE 6.—Halo length, seed-surface area, seed volume, and boll volume. Halo length represents the average of 10 seeds with 6 measurements each around the median region of the long axis. Seed-surface area is the average of 25 seeds, seed volume of 40 seeds; and boll volume of 10 bolls.

differential growth rate must be the basis for the physiological explanation of the fiber arrays with all their variations. A study of sorter graphs such as that for 50-day-old bolls in figure 8 suggested to the writers that perhaps each of the 24 to 25 groups may have originated from the seed surface at approximately equal intervals of time, i.e., the longest fibers may have arisen during the first few days of relatively small surface area, the bulk of the fibers represented by the larger blocks may have arisen during the period of rapid expansion in surface area to about 21 days (fig. 6), and the short fibers may have arisen during the period of declining expansion in surface area. Such an explanation is based on the assumption that

there is a continual differentiation of epidermal cells for many days after flowering. An article by Turner (24), received at the end of the first season's work, contained evidence of such a continuous differentiation. Gulati (15), Singh (22), and Farr (14) have likewise presented evidence which makes it appear that such is the case, though Balls (6) was not convinced by the evidence of Turner or Gulati and still holds that his earlier statements are probably correct. Barritt (7, 8) also is not convinced that there is a continuous differentiation of epidermal cells.

In 1930, efforts were directed chiefly to obtaining evidence that might refute or substantiate the assumption that the different length groups arose from the seed surface at different times, and that factors acting at different stages of boll development might thereby produce the greatest effect on those groups of fibers in the more active phase of elongation with the resultant variations to be observed in the sorter arrays.

GROWTH OF THE SEED IN SURFACE AREA AND VOLUME

Fifteen hundred blooms were tagged on 1 day at approximately the same branch and node to give material thereafter for 30 daily measurements of seed-surface area, seed volume, and boll volume. The seeds for study were taken from the middle position in the locules of 10 bolls which were collected daily. The fiber was removed, and the length and breadth of the seed measured. The removal of the fiber was difficult for the first few days, but soon it could be performed so that the volume displacements in paraffin oil were fairly accurate. The cottonseed is practically a prolate spheroid, and the formula $2\pi b^2 - 2\pi \frac{ab}{2} \sin^{-1} e$ was used for calculations of surface area, where a is one half of long axis, b is one half of short axis, and e is the eccentricity. The data for seed from 4 to 30 days of age are given in the following tabulation:

Age, in days	Surface area, in square millimeters	Age, in days	Surface area, in square millimeters	Age, in days	Surface area, in square millimeters
4	16.6	14	137.7	23	220.2
6	25.6	15	142.9	24	225.5
7	33.2	16	177.5	25	238.5
8	41.6	17	166.6	27	232.4
9	47.7	18	196.3	28	232.1
10	54.7	20	201.9	29	232.4
11	78.4	21	223.1	30	241.5
13	101.7	22	217.7		

25 seed measured each day.

The curve for surface area is shown in figure 6. It is seen that the surface area of the seed at 30 days is 14.5 times that at 4 days, which of itself might suggest an increase in the number of fibers with increasing age. Much of the work of Farr (14) was done in the same laboratories on material grown from seed obtained from the same source and planted in adjoining plots which received similar treatment. The measurements for transverse axes of epidermal cells given by Farr in table II of her paper can, therefore, be used to

calculate the number of epidermal cells per seed at various ages. If her figures of 67,400 epidermal cells at 1 day of age are used, the number for the writers' material will be 409,236 at 7 days, 526,670 at 13 days, and 676,121 at 20 days. Though the transverse axis of the epidermal cell is increasing with age, it is not increasing so rapidly as the increase in surface area, as is indicated by these figures and shown in the illustrations of dividing epidermal cells presented by Gulati (15) and Farr (14). Turner (24), in his table I, gives the totals of fiber and ordinary cells on the 1-day-old seed for two Punjab-American cottons as approximately 27,000 and 28,000. The number of fibers approximates 6,000 at 1 day and 25,000 at 28 days. The number of fibers per seed shown in table 4, calculated by a method which is believed to be more accurate, is only 11,807. It should be noted that the numbers of epidermal cells according to the various calculations, which are only approximations, are therefore much greater than the usual count of fibers per seed, indicating that the majority of the cells may finally produce only fuzz. Barritt (8) contends that his measurements for increase in surface area of the epidermal cells agree remarkably well with Farr's figure for the increase in surface area of the ovule, and that the evidence from cell measurements thus does not support the possibility of continuous cell division in the epidermis.

The seeds used for surface-area measurements were also used for the determinations of volume. Forty of the delinted seeds were dropped into a calibrated pipette containing paraffin oil, and the displacement noted. The curve in figure 6 gives some idea of the changing relations of surface area for the production of fibers and the volume of material stored in the seed. A comparison of the curves for boll volume and seed volume indicates that after 10 days, when the boll cavity becomes filled with developing seed and lint, the relative increase in volume of the two structures is about the same.

HALO LENGTH

Balls (3) and Brown (9) have measured the halo lengths of Egyptian cottons, while Hawkins and Serviss (16) have measured those of Acala, an American upland, and Pima, an Egyptian-American cotton. The halo length of Super-Seven was taken in order to determine more definitely the period when maximum length was attained. The fibers were measured on one seed from the same position in a lock of each of 10 bolls. The seeds were floated in paraffin oil and an attempt was made to allow the fibers to assume their natural position as nearly as this could be determined. The fibers when combed almost completely surrounded the seed. No large angle of the wings with the longitudinal axis was formed, as shown by Bailey (2), and the writers' method, therefore, differed somewhat from that described by him. Twelve measurements were made of the fibers on each seed, 3 around the pointed end, 3 around the large end, and 3 on each side. It is evident from figure 6 that the growth in length was practically complete in 25 days, a period in agreement with that found by Balls (3) for Egyptian cotton. Apparently, however, full length in Super-Seven had not been reached in 30 days, as compared with 21 and 24 days for practically complete length in Acala (16).

FIBER ARRAYS OF IMMATURE BOLLS

Preliminary but unsuccessful attempts were made to count the number of fibers per seed at daily intervals by the method that Gulati employed in Turner's (24) laboratory. Gulati's (Turner (24)) observation of the increasing number of fiber cells on the seed surface from 1 to 28 days and Farr's (14) observation of the early stages of fiber elongation from epidermal cells, which were apparently daughter cells of recent division, would seem to indicate a distinct probability that the shorter lengths represented in the array actually did arise at later stages than the longest fibers measured as the halo. The assumption previously made (p. 466) that the 24 or 25 length groups of the sorter array may have originated from the seed surface at approximately equal intervals and that each group may have reached its length under the same approximate conditions must, of course, be accepted only as an assumption, for a weight distribution may not be a very accurate indication of the number of fibers in a group. Iyengar and Turner (19) show that the length distribution by number of fibers is only approximately represented by the sorter distribution by weight, though the divergence is not great for the varieties of *Gossypium hirsutum* which they investigated. The number-frequency distribution of the fibers of a Texas cotton shown by Clegg (11) is quite similar, however, to the weight distributions as presented in this paper. Clegg states that the effective length found from the number-frequency curve differs from that found from the weight frequency by not more than about 3 percent with the usual cottons, on the assumption that there is a constant fiber weight per centimeter. This assumption is not correct for many cottons, as shown by Turner (24), Iyengar and Turner (19), Campbell (10), and the present writers (table 4).

In order to compare the weight-frequency distribution with a number-frequency distribution, the number of fibers per seed was determined. Three hundred fibers from each length group of an array, if so many were available, were weighed and the number of fibers was calculated for each group. This was done for 11 bolls on one plant (table 3). The array from this number-frequency distribution is compared with the weight-frequency distribution in figure 7. It is seen that the percentage of fibers in the short lengths, especially two, three, four, and five sixteenths, and even to ten sixteenths, is relatively much greater in the number-frequency distribution than in the weight-frequency distribution. Tips of fibers supposedly break with greater ease than other portions, and broken lengths might account for the greater number of fibers in the short lengths. To test this point, 535 fibers from several lengths were mounted in small groups in paraffin oil and examined microscopically at both ends. One can be fairly certain about broken tips on longer fibers, but there is some difficulty in deciding the condition of fibers two sixteenths of an inch in length by the method employed. Of 257 fibers in the eighteen and twenty-two sixteenths groups, 10.9 percent had broken tips and 14 were unclassified. Of the 165 examined in the two sixteenths group, 39 percent were broken and 24 were unclassified. If the 24 unclassified fibers are placed in the broken group, the percentage is raised from 39 to 53.6. It is entirely possible that portions of the tip less than two sixteenths of an inch are broken and thus lost for an array. The

examination of the long fibers leads to this suspicion, though it is difficult to prove. Not very many of the fibers in the two-sixteenths group appeared to be broken tips, however, for the broad, thicker fibers, characteristic of the shorter lengths were most evident. Whatever may have been the source of the broken fibers, it appears that relatively more of them are in the very short groups, and if these could be distributed into their proper places the weight-frequency and number-frequency histograms would more nearly coincide. Thus it

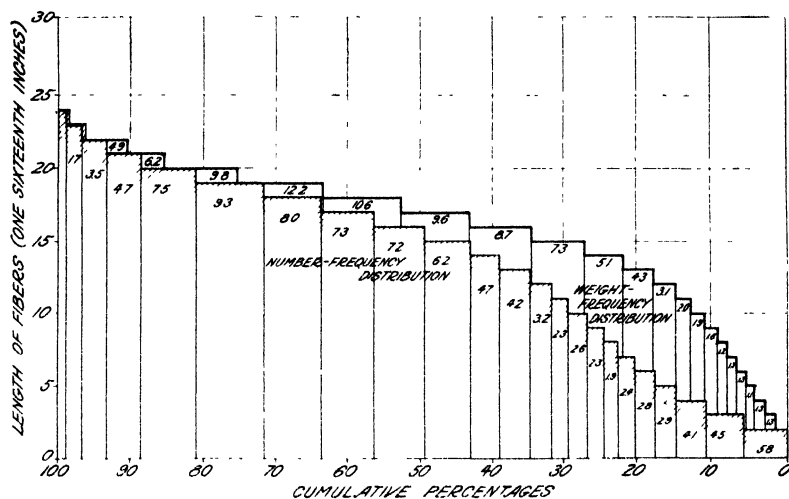


FIGURE 7 --Average weight- and number-frequency distributions of the fibers per seed from 11 bolls of one plant

appears that the weight-frequency sorter graph can be used as a fair measure of the number of fibers concerned, except perhaps in the very short groups.

TABLE 3.—Average number of cotton fibers per seed from 11 bolls on 1 plant

Lengths, in sixteenths of an inch	Average number of fibers per seed	Per-centage of total	Lengths, in sixteenths of an inch	Average number of fibers per seed	Per-centage of total	Lengths, in sixteenths of an inch	Average number of fibers per seed	Per-centage of total
27	0 3	-----	17	905 9	7 20	7	331 7	2 43
26	3 9	0.03	16	978 9	7 17	6	378 2	2 77
25	46 4	.34	15	848 8	6.21	5	394 8	2.80
24	113 3	.83	14	638.5	4.67	4	557.2	4 08
23	234 3	1.72	13	572 3	4 19	3	607 7	4 45
22	481.7	3.63	12	437 1	3.20	2	785 7	5 75
21	645 1	4.72	11	312.5	2 20			
20	1,026.5	7.51	10	347 7	2.55	Total	13,661 1	-----
19	1,265 1	9 26	9	312 9	2.20			
18	1,080.0	7.97	8	255.6	1.87			

The sorter array of fibers gives an opportunity for counting the number of fibers per seed, but such an array would scarcely be possible at very early stages in boll development. An attempt was made, however, to array fibers at approximately 4-day intervals from bolls 18 to 50 days of age. Ten-boll samples of the different ages were collected at one time. These were air-dried in the laboratory for

2 days and then placed in an oven at very low heat until all that would open had done so. The 50-day-old bolls picked in the morning had cracked by the first afternoon in the laboratory. Fibers from all bolls under 25 days of age were unsatisfactory for an array. Juices flowed from the bolls and the dry mass of fibers crumbled when handled. The fibers from bolls 25 days of age could be prepared for the array with almost the same ease as those from bolls that opened naturally. Fiber arrays for seven samples are represented in figure 8. This figure shows that halo measurements of fibers from bolls 25 days old would be practically the same as those from bolls 50 days old, but that the proportion of fibers 1 inch or longer is only 29.22 percent in the 25-day bolls, whereas in the 50-day bolls it is 80.59 percent.

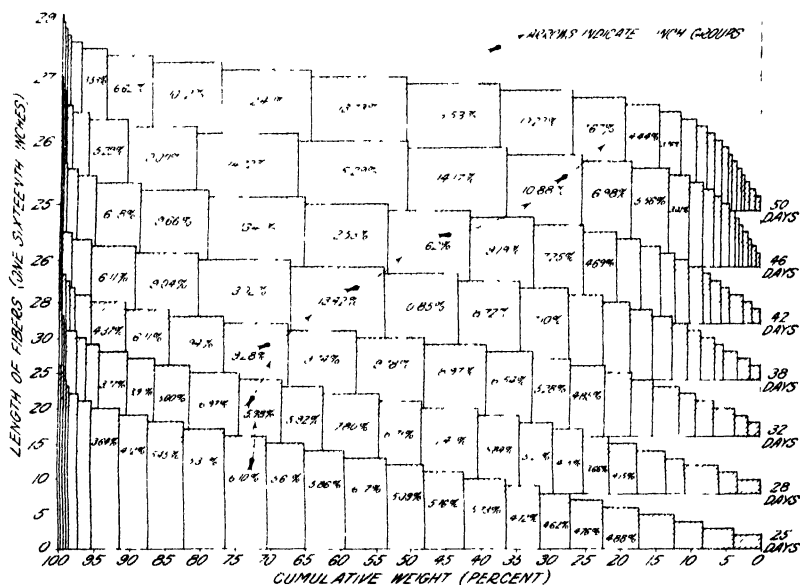


FIGURE 8.—Sorter arrays of the lint from bolls of different ages. Each histogram represents an average of the arrays of 10 bolls. Figures are percentages.

There seemed to be a distinct possibility that more fibers might be broken from a boll 25 days of age than from older ones. Therefore, microscopic observations of 835 fibers from this array, 577 from the array of 38-day-old bolls, and 535 from the array of 46-day-old bolls were made, showing 28, 23, and 22 percent broken fibers, respectively. The relatively small difference in the percentage of broken fibers does not explain the decided differences in the percentages of the total weight in the various length group.

If it is assumed that no further appreciable increase in length of any fibers will occur after 25 days and that the number of fibers per seed is always the same, the change in the distribution of fibers from the 25-day-old boll to that of the 50-day-old boll might conceivably be brought about by a different fiber weight per inch in the process of wall thickening. The curve in figure 6 for fiber growth in length indicates the usual S-shaped growth curve. If it is assumed that some further extension in length of fibers may occur after 25 days, contrary

to the beliefs of Balls (3, 4) and Hawkins and Serviss (16), it appears possible that the short fibers which may have arisen at various stages of seed development may continue to grow and reach the final length already attained by others and thereby give the distribution of the 50-day-old boll. If one accepts Balls' (3, 4) statement that the fibers arise on or near the day of blooming, the further extension in length would occur in fibers whose growth had been checked. A changing fiber weight per inch and a further growth in length of some fibers may therefore be important in producing the final result attained. This latter point is the most difficult one on which to secure direct experimental evidence. The unit fiber weight per inch and the number of fibers per seed, however, have been secured for bolls of 25, 32, and 50 days of age. The data for the first and last ages only are shown (table 4).

TABLE 4.—Number of fibers per seed and the unit fiber weight for cotton bolls 25 and 50 days of age

Length in sixteenths of an inch	Fibers counted 25 days old	Fibers counted 50 days old	Fibers per seed 25 days old	Fibers per seed 50 days old	Weight per inch of fiber 25 days old	Weight per inch of fiber 50 days old	Ratio of weight, 50 days 25 days
	Number	Number	Number	Number	Milligram	Milligram	
26		19		3 8			
25	48	52	5 3	18 4	0 .0013	0 .0032	2 .46
24	50	130	7 7	83 4	.0013	.0035	2 .69
23	50	260	38 8	316 2	.0014	.0040	2 .86
22	50	300	77 2	701 8	.0015	.0039	2 .60
21	72	300	84 5	1,094 0	.0018	.0038	2 .11
20	250	300	163 3	1,282 0	.0024	.0043	1 .79
19	286	300	220 5	1,406 4	.0021	.0045	2 .14
18	350	300	346 4	1,504 4	.0021	.0049	2 .33
17	350	300	420 0	1,109 2	.0023	.0052	2 .26
16	350	300	610 6	820 6	.0025	.0054	2 .16
15	350	300	661 8	429 2	.0022	.0057	2 .59
14	350	300	695 8	400 2	.0023	.0057	2 .48
13	350	300	771 3	286 2	.0025	.0059	2 .36
12	350	300	663 1	210 6	.0021	.0057	2 .38
11	350	300	906 4	187 4	.0025	.0056	2 .24
10	350	300	935 2	198 8	.0025	.0058	2 .32
9	350	295	830 1	191 4	.0027	.0053	1 .96
8	350	300	950 2	111 6	.0028	.0059	2 .11
7	350	300	705 5	188 6	.0029	.0063	2 .17
6	350	300	694 1	184 2	.0031	.0060	1 .94
5	350	300	916 8	204 4	.0031	.0059	1 .90
4	350	300	906 6	265 6	.0042	.0076	1 .81
3	350	300	1,685 .2	231 6	.0043	.0100	2 .33
2	350	300	1,449 0	308 0	.0055	.0080	1 .45
Total.			14,814 .4	11,807 0			

The number of fibers per seed for 25- and 50-day-old bolls was obtained from triplicate arrays of the fiber of 9 and 5 seeds, respectively, from 3 different bolls. A total of 350 fibers from each length, if available, was counted for the 25-day-old bolls, and 300 fibers from each length for the 50-day-old bolls. The average number of fibers per seed for the ages 25, 32, and 50 days was 14,814.4, 14,266, and 11,807, respectively, thus showing considerable variation without an indication of an increasing number of fibers per seed after 25 days of age. The curve for growth in surface area of the seed shown in figure 6 would not lead one to expect a large increase in the number of fibers, though the increasing surface area to 30 days might produce other fibers.

An examination of the evidence concerning the possibility that a changing fiber weight per inch during development might cause the difference in the fiber arrays from bolls 25 and 50 days of age, as presented in table 4, shows that the fiber weight per inch at 50 days averages 2.23 times the weight at 25 days. To produce such changes as are shown in the sorter arrays, however, it would be necessary for the medium and longer length groups to increase in weight relatively much more than the shorter ones. While such a tendency is indicated by the figures in the last column of table 4, it is in nowise sufficient to produce the marked change noted from the array. Calculations of the fiber weight per inch from a number-frequency distribution rather than a weight-frequency distribution do not materially change the situation.

Since the changing fiber weight per inch for different lengths does not explain the change in arrays of fibers from bolls 25 to 50 days of age, a growth in length of some fibers after 25 days appears a reasonable explanation. This is supported by the extension of the halo length to 30 days, as shown in figure 6.

The inability to count fibers before the seeds are 25 days old leaves the writers without direct evidence concerning the controversial subject of a continuous differentiation of fibers after the day of blooming, and likewise without direct evidence as to the truth or falsity of the assumption that the different length groups of the sorter array may have originated at different intervals. It seems clear from figure 8, however, that contrary to prevailing ideas, there must have been an increase in the length of many fibers in the boll after 25 days of age.

Because of the evidence for an increasing number of fibers per seed with increasing age to about 28 days (15, 24), the presence of dividing epidermal cells on the outer seed coat (14, 15), fibers of various lengths, side by side at different ages of the seed (14, 15, 22), increase in surface area of seed greater than increase in length of transverse axes of epidermal cells (14), and the finding here reported of an increase in the length of fibers in the boll after 25 days of age, the authors are inclined to believe that this increase in length occurred in fibers which originated from the seed surface at different intervals.

SUMMARY

Cotton fibers from all bolls on individual plants have been arrayed by the use of Baer and Johannsen sorters which allow an accurate separation of the different lengths into groups by weight.

A difference of six sixteenths of an inch in the practical staple length of the cotton classer has been found between the fibers of two bolls located rather close together on a plant. Very appreciable differences in the uniformity of distribution of the fibers of various lengths have also been shown.

In terms of the staple length of the cotton classer, a difference of three sixteenths of an inch in length was found between the lint from seed of one locule and the same difference between the lint from bolls developing from blooms of the same day. Five sixteenths of an inch was the difference in length of lint from blooms of two successive days.

Conditions seemed to be favorable for the production of long lint in bolls from all flowers during certain periods of time. During certain

short intervals, however, conditions seemed very unfavorable, as relatively short lint was produced.

The position of the boll along the vertical axis or the horizontal axis does not seem to be of very great importance in determining the length of lint, though there is a distinct tendency for shorter lint to be produced near the top of the plant when grown in the field.

Studies have been made of halo length, seed-surface area and volume, boll volume, number of fibers per seed after 25 days of seed development, unit-fiber weight, and sorter distribution of fibers in bolls at approximately 4-day intervals from 25 to 50 days of age.

The increase in surface area of the seed was most rapid from about 10 to 18 days of age.

The relative increase in seed volume and boll volume was about the same after 10 days, when the boll cavity becomes filled with developing seed and lint.

The halo had practically reached full length in 25 days, though not absolutely so in 30 days.

There was no evidence of an increase in the number of fibers per seed after 25 days.

The unit-fiber weight was not constant, the shorter fibers showing the greatest weight per inch.

The sorter distributions of fibers from bolls at approximately 4-day intervals from 25 to 50 days of age indicate that many of the fibers continue to elongate after the boll is 25 days old.

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EFFECT OF SOIL FERTILITY, BOLL-MATURATION PERIOD, AND EARLY OR LATE PRODUCTION OF BOLLS ON THE LENGTH OF COTTON FIBERS¹

By G. M. ARMSTRONG, *botanist and plant pathologist*, and C. C. BENNETT, *research assistant*, South Carolina Agricultural Experiment Station

INTRODUCTION

In a previous paper² the influence of certain factors on the variability in the length of cotton fibers on individual plants has been presented. The plants to be considered in this paper were grown along with those previously described. The details of plot treatment and other experimental methods are found in the first publication, and need not be repeated here.

EFFECT OF SOIL FERTILITY ON LENGTH OF FIBER

One part of the experimental field was given liberal applications of phosphorus, potash, and nitrogen, and the other part was given none. Otherwise cultural conditions were the same on both. The growth of the plants on the well-fertilized or high-fertility plot was apparently normal, and the plants compared quite favorably with those on other high-yield plots at the experiment station. In 1929 the plants on the unfertilized plot began to show signs of a nitrogen shortage relatively early, and finally the symptoms of an unbalanced nutritional condition were strikingly apparent, the deficiency of potash being most clearly evident. On September 14 an appreciable shedding of leaves was noted, which continued until about the middle of October, when frost caused the loss of all leaves. Some shedding of leaves, though of decidedly lesser proportions, also occurred in the well-fertilized plot.

One hundred blooms opening on August 14 and 15 at the first node of branches 1, 2, and 3 were tagged on each plot. On August 21 a larger number of blooms were tagged at the first node on branches 3, 4, and 5 on the unfertilized plot and at branches 4 and 5 on the fertilized plot.

The average arrays of the fibers from 16 bolls and 10 bolls tagged August 14 and 15 from each of the fertilized and unfertilized plots showed a difference in lint length of less than one sixteenth of an inch, except for some of the shorter fibers (fig. 1, A). Average arrays of the fibers from 10 and 25 bolls tagged August 21 from each of the fertilized and unfertilized plots were also made. Since the 10-boll and 25-boll arrays checked closely, only the curves for the latter are shown (fig. 1, B).

¹ Received for publication Sept. 9, 1932; issued October, 1933. Technical contribution no. 30 (new series) from the South Carolina Agricultural Experiment Station

² ARMSTRONG, G. M., and BENNETT, C. C. SOME FACTORS INFLUENCING THE VARIABILITY IN LENGTH OF COTTON FIBERS ON INDIVIDUAL PLANTS AS SHOWN BY THE SORTER METHOD. *Jour. Agr. Research* 47: 447-466. 1933.

The same plots were used in 1929 and 1930, and similar comparisons were made for both years. No greater differences were observed, however, than those shown in figure 1, A and B. It will be seen that

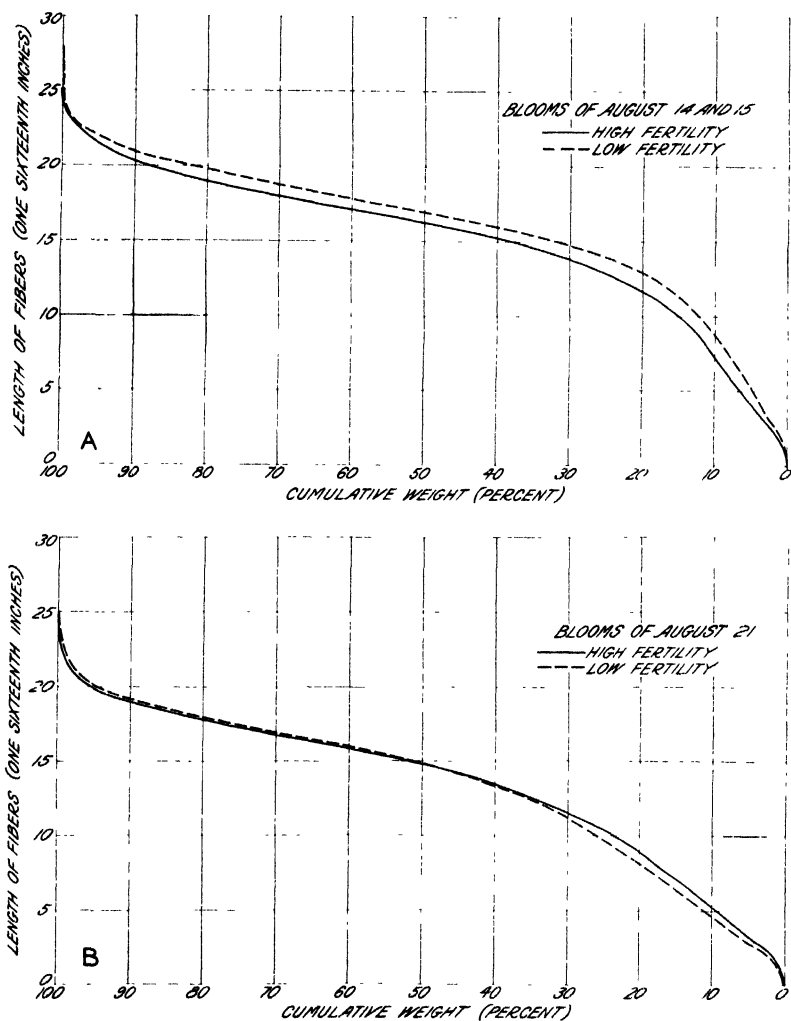


FIGURE 1.—A, sorter arrays of the lint from 16 bolls grown on a high-fertility plot and 10 bolls grown on a low-fertility plot, B, sorter arrays of the lint from bolls grown on high- and low-fertility plots, each curve representing an average of the arrays of 25 bolls

the lint length of the cotton grader was practically the same for both samples. The length of the lint was not therefore materially changed by the contrasting treatments, in spite of the very unfavorable conditions for growth in the unfertilized plots, which resulted in stunted and unhealthy plants often bearing only 1 or 2 bolls. Sturkie³ states

³ STURKIE, D. G. A STUDY OF SOME OF THE FACTORS AFFECTING LINT DEVELOPMENT IN COTTON. Ala. Agr. Expt. Sta. Ann. Rpt. 41: 19 1930.

that length of lint and size of boll were the same from plants grown under optimum moisture conditions on a fertile Mississippi Delta soil and a Norfolk sand.

In 1929 all the bolls in this particular phase of the work were marked with India ink and measured twice daily with calipers. The diameter measurements were converted into volume on the assumption that the boll is a sphere, the formula $V = 4.189r^3$ being used. Volume-displacement measurements made on 10-boll samples show that the calculated volumes are about 5 and 6 percent less than the actual volumes until the boll is 7 to 9 days old and that the divergence increases thereafter until the calculated volumes are 13 to 16 percent less when the boll reaches full size. A volume measurement based on a 10-boll sample is only approximate, and because of the difficulties of securing and measuring the large number of bolls necessary for accurate determinations of volume, the calculated volumes are used. In contrast with the results of Sturkie,⁴ table 1 shows that there were differences in the size of bolls from well-fertilized and unfertilized plots, the bolls from the latter being smaller. The bolls in this experiment were grown under moisture conditions far from the optimum, as compared with those prevailing in Sturkie's experiments, where boll size was unaffected by low soil fertility. There were days when the plants in the field wilted noticeably during the dry weather. Thus boll size was decreased on the low-fertility plot without materially shortening the lint.

TABLE 1.— *Size of bolls from cotton plants grown on fertilized and unfertilized field plots*

Plot	First series, blooms of Aug 14 and 15				Second series, blooms of Aug 21			
	Bolls	Age at maximum volume	Maximum volume	Final volume	Bolls	Age at maximum volume	Maximum volume	Final volume
	Number	Days	<i>MI</i>	<i>MI</i>	Number	Days	<i>MI</i>	<i>MI</i>
Fertilized..	16	34	31 62±1 14	30. 32±1 14	40	36	32 16±0 66	31 04±0 64
Unfertilized..	13	33	26 48± 90	23 24± 88	71	37	28 72± 15	27 05± 47

Statistical analyses of boll-size measurements in connection with other phases of the work have shown that results based on 13 or 16 bolls alone should be accepted as doubtful, but the results presented in table 1 for the first series agree with those of the second series with its larger number of bolls and are believed to be indicative of the true situation. A 50-boll sample measured for size gives fairly reliable results, though for a greater degree of accuracy a 100-boll sample should be used.

EFFECT OF AGE OF BOLLS ON LENGTH OF FIBER

It has been noted ⁵ that bolls on a single plant from blooms of 2 successive days show appreciable variations in length and uniformity of lint, though there is a distinct tendency for lint in all bolls to be

⁴ STURKIE, D. G. (See footnote 3.)

⁵ ARMSTRONG, G. M., and BENNETT, C. C. (See footnote 2.)

longer or shorter during certain periods. The relation of date of blooming to length of lint was noted in the field in 1929 by tagging 100 blooms at comparable positions on plants in both fertilized and unfertilized plots on August 14 and 15 for the first series and on August 21 for the second series. Fibers from 16 bolls of the first series and 27 bolls of the second series from the fertilized plot were

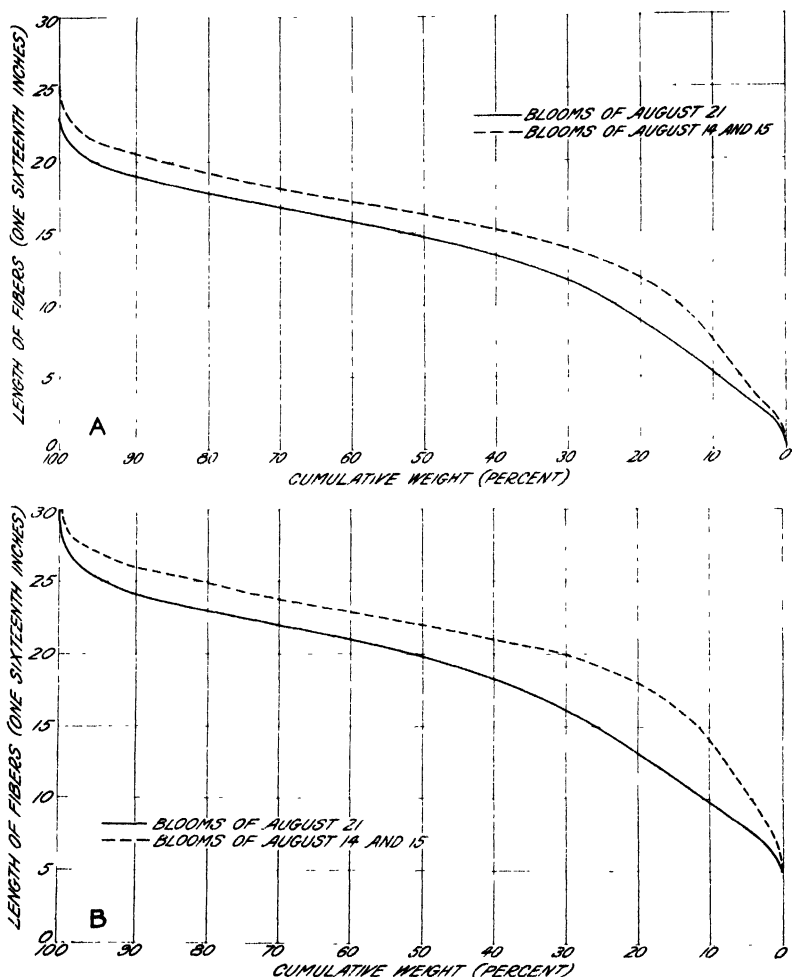


FIGURE 2—A, Sorter arrays of the lint from bolls of different ages grown on a high-fertility plot using an average of 16 bolls August 14 and 15, and 27 bolls August 21; B, sorter arrays of the lint from bolls of different ages grown on a low-fertility plot using an average of 10 bolls August 14 and 15, and 25 bolls August 21.

arrayed. Curves representing an average of the arrays are shown in figure 2, A. It is evident that the percentage distribution of the group lengths has been affected by the age of the bolls and that the later developing fibers are shorter by at least one sixteenth of an inch in terms of the cotton classer. Fiber arrays of 10 bolls of the first series and 25 bolls of the second series from the unfertilized plot are shown

in figure 2, *B*. It is evident that the percentage distribution of the group lengths has been affected by the treatment, for the later developing fibers are almost two sixteenths of an inch shorter in terms of the cotton classer.

That a water shortage may decrease lint length has been shown by Balls⁶ and Kelsick⁷. Sturkie⁸ states in an annual report, without supporting data, that low soil moisture 1 to 15 days after blooming is associated with short lint. It is also a common belief among cotton growers that the lint length of standard varieties is shorter during dry seasons. An examination of the rainfall and soil-moisture records given in table 2 indicates a probable relation between decreasing soil moisture and the shorter length of lint in the series of August 21. This latter series was subjected to rather low soil moisture for the first 25 days of development, to September 16. At this age bolls have practically reached full size and much of the growth in length of the fibers has been attained.

TABLE 2.—*Rainfall and soil moisture at Clemson College, S.C., for a part of the cotton-growing season of 1929*

Date	Rainfall	Soil depth	Moisture	Date	Rainfall	Soil depth	Moisture
	<i>Inches</i>	<i>Inches</i>	<i>Percent</i>		<i>Inches</i>	<i>Inches</i>	<i>Percent</i>
Aug 7 -	0.02			Sept. 7 -		6	12.5
9 -	0.05			8 -		18	14.5
12 -	1.00			10 -	0.19		
17 -	.10			11 -	.02	6	5.0
21 -		6	2.6	13 -		18	9.0
23 -	.10	18	5.1	14 -	.45	6	6.9
24 -		6	7.5	16 -	.87	18	7.6
27 -		18	19.0	17 -	1.30		
31 -		6	2.9	22 -	.34		
Sept 4 -	.78	18	15.3	23 -	Trace		
6 -	.02	6	3.7	25 -	2.51		
		18	12.7	26 -	1.30		
			1.6				
			13.0				

* 18-inch sample always a heavy clay

EFFECT OF PERIOD OF MATURATION OF BOLLS ON LENGTH OF FIBER

The date of blooming was used as the basis for the collection of practically all samples because of the fact that some of the most critical stages in boll development occur during the early part of its development and that there are decided differences in the length of the maturation period of different bolls. Burd⁹ is of the opinion that bolls which open at about the same time should be used for the comparison of strains of cotton. Balls¹⁰ has shown that the lint lengths are much more irregular if the cotton is gathered by the date of opening of the boll rather than by the date of flowering. Ayyar

⁶ BALLS, W. L. THE DEVELOPMENT AND PROPERTIES OF RAW COTTON 221 p., illus. London. 1915

⁷ KELSICK, R. E. SOME OBSERVATIONS ON THE RELATIONS OF LINT LENGTH TO RAINFALL. West Indian Bul. 17: 79-82, illus. 1920.

⁸ STURKIE, D. G. (See footnote 3.)

⁹ BURD, L. H. REPORTS ON THE RESEARCH WORK CARRIED OUT AT THE COTTON EXPERIMENT STATION, ST. VINCENT, WEST INDIES. Emp. Cotton Growing Rev. 2: 225-228, illus. 1925.

¹⁰ BALLS, W. L. (See footnote 6.)

and Rao ¹¹ have shown for both *Gossypium hirsutum* and *G. indicum* that definite differences existed in the lint length of seeds produced in different pickings but that there was no definite relation between lint length and the date of picking.

The material from the unfertilized plot offered some distinct contrasts in the maturation period of bolls and two samples have been arrayed. Twenty-four bolls that developed from blooms of the same date and in comparable positions on the plants were selected and divided into two groups of 12 each. The group with the relatively short maturation period contained bolls that opened from 55 to 63 days, the average maturation period being 58 days. The group with the relatively long maturation period contained bolls that opened from 68 to 91 days, the average maturation period being 75.1 days. An inspection of figure 3 shows that the later-opening bolls had more short fibers, and therefore a less uniform staple, but that the practical staple

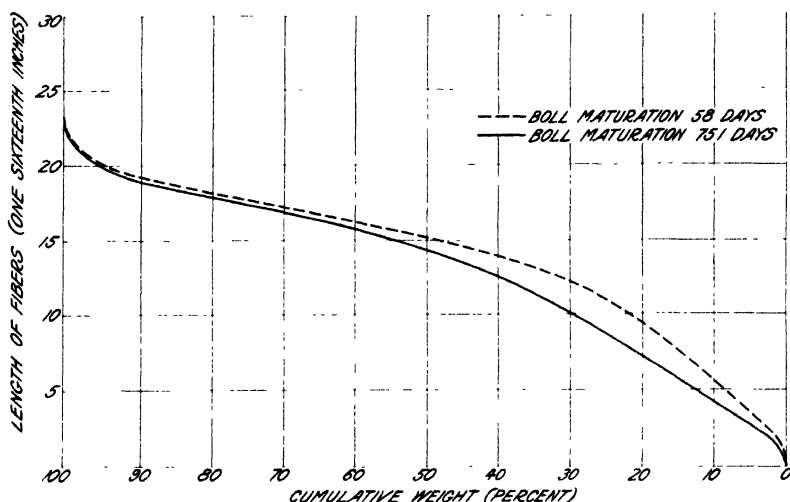


FIGURE 3 Sorter arrays of the lint from bolls with different maturation periods but arising from flowers of a single day, each curve representing an average of the arrays of 12 bolls

length was the same. It has been noted previously that the plants on the unfertilized plot were small and that there were very evident signs of unbalanced nutritional conditions, especially potash hunger, accompanied by some defoliation.

RELATIVE LENGTH OF FIBER IN FIRST AND SECOND CROPS OF BOLLS

The branches and main stems of cotton plants in the greenhouse practically ceased to elongate during the development of the first load of fruit. After most of the first crop of cotton was picked, new growth began at the tips of branches and the main stems, and axillary buds also developed into branches which bore fruit. Since this growth

¹¹ AYYAR, V. RAMANATHA, and RAO, C. JAGANNATHA. VARIATION IN LINT LENGTH IN COTTON. Agr Jour. India 25: 42-52, illus. 1930.

in the fall and winter occurred under conditions different from that of the main crop, especially with reference to temperature and length of day, a second crop of bolls was allowed to mature and the cotton was picked chiefly in December, January, and February.

Plant no. 13 produced only 6 bolls as the second crop. The lint from 4 of these would probably be classed as nineteen sixteenths of an inch and that from the other 2 as twenty sixteenths. The lint was neither as long nor as short as that produced in some of the 50 bolls of the first crop. The first crop from plant no. 2 consisted of 20 bolls and the second crop consisted of 11. The average lint length

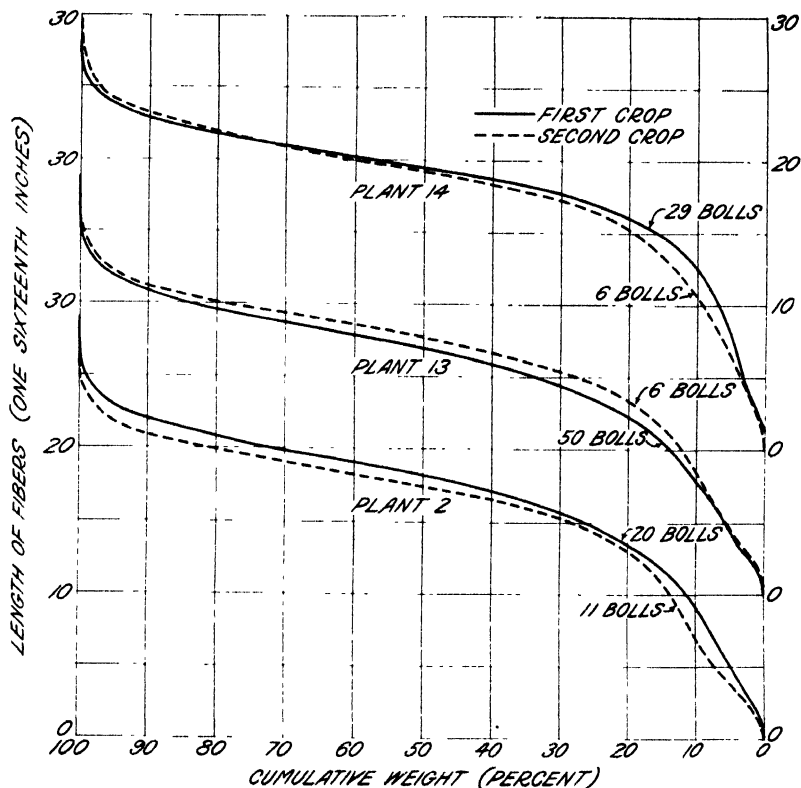


FIGURE 4.—Sorter arrays of the lint from bolls of the first and second crops on plants in the greenhouse.

of the 20 bolls of the first crop was twenty sixteenths of an inch and that of the 11 bolls of the second crop was nineteen sixteenths. The range of variation in lint length from individual bolls of the first crop was seventeen to twenty-one sixteenths of an inch and from bolls of the second crop, seventeen to twenty sixteenths. These illustrations, together with the data presented in figure 4, show that the lint from bolls of the second crop was similar in length to that from bolls of the first crop and that the distributions of the different length groups also maintained about the same relations.

SUMMARY

Small plants, growing on plots of low fertility, and clearly suffering from malnutrition, produced lint of practically the same length as that produced by vigorous plants growing on plots of high fertility, though the uniformity of distribution of the different lengths was less in the poorly nourished plants.

The lint from bolls produced from blooms that were among the last to be retained, namely, those of August 21, was from one sixteenth to two sixteenths of an inch shorter than that from bolls arising a week earlier, on August 14 and 15.

Bolls from blooms opening on the same day but with a maturation period of 75.1 days had a larger percentage of short fibers than those with a maturation period of 58 days, though the practical lint length was the same.

The variability in lint length and the distribution of the different length groups was about the same in bolls of a first crop and in those of a second crop produced on the same plants.

THE NATURE OF THE SHEATH MATERIAL IN THE FEEDING PUNCTURES PRODUCED BY THE POTATO LEAF HOPPER AND THE THREE-CORNERED ALFALFA HOPPER¹

By FLOYD F. SMITH²

Formerly associate entomologist, Division of Cereal and Forage Insects, Bureau of Entomology, United States Department of Agriculture³

INTRODUCTION

In a histological study of plant tissue injured by several species of leaf hoppers of the genus *Empoasca*, Smith and Poos (19)⁴ showed that the feeding of each species was restricted to certain tissues, the mesophyll or the vascular bundle (phloem and xylem), and that the resulting injury to the host was correlated with the parts fed upon. The mesophyll-feeding species of *Empoasca* caused a stippling of the leaves, whereas the species feeding on the vascular tissue, *E. fabae* (Harris),⁵ produced severe types of diseaselike injury on its various hosts. In the feeding punctures produced by all species, an amber-colored material that stained bright red with safranin was found either in the form of a cylindrical sheath about the punctures made by the insect's mouth parts or in aggregations at the ends of the punctures. This material was present in both new and old punctures. Smith and Poos suggested that its presence in the vascular cells might interfere with the process of translocation of plant materials and cause the plant to wilt when the xylem vessels were plugged or to become red or yellow when phloem tissue was fed upon.

In order to obtain further knowledge of the injury caused to plants by these insects, particularly by *Empoasca fabae*, it seemed desirable to determine the source of the sheath material and whether it is produced by the plant as a result of the puncture, by the leaf hopper alone, or by both the insect and the plant. The tests were made on sheaths surrounding feeding punctures made by adults of *E. fabae* and also, for comparative purposes, on those made by the adult three-cornered alfalfa hopper, *Stictiocephala festina* (Say)⁶. The material was found in greater abundance in its feeding punctures than in those of *E. fabae*. The substances for which tests were made include those that are found in the cell walls and are formed by the plants, as well as additional substances.

REVIEW OF LITERATURE

The literature contains several references to sheaths in the feeding punctures of sucking insects, but investigators have differed in their conclusions as to the nature and source of this material. Prillieux

¹ Received for publication May 31, 1933; issued October, 1933. This paper presents the results of one phase of a cooperative study of leaf-hopper injury to forage crops undertaken by the Division of Cereal and Forage Insects, Bureau of Entomology, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

² The writer is indebted to F. W. Poos for making possible the study of this problem, to H. O. Sampson, Mary E. Reid, and H. W. Johnson for advice and suggestions; and to J. W. Schrivener for assistance in preparing the insect-injured material used in the tests.

³ Now entomologist, Division of Truck Crop and Garden Insects, Bureau of Entomology, U. S. Department of Agriculture.

⁴ Reference is made by number (italic) to Literature Cited, p. 484.

⁵ Order Homoptera, family Cicadellidae.

⁶ Order Homoptera, family Membracidae.

(17, p. 43), in his studies of the feeding punctures made by *Puceron* (= *Eriosoma*) *lanigerum* Hausm. in apple in 1878, was apparently the first to describe the sheath in plant tissue around the proboscis of an insect; he believed that the sheath material was analogous to cellulose. Davidson (4) stated nearly a half century later that aphid sheaths were probably composed of callose or cellulose and tannin; and more recent tests by Painter (15, pp. 234-237) suggested the presence of callose, calcium pectate, and tannins in sheaths caused by punctures of the chinch bug, *Blissus leucopterus* (Say). These results indicate that the sheath was produced by the plant cells. Smith (20, pp. 118, 120, 131) believed that the sheath was made up of substances of both plant and animal origin, but he made no tests. King and Cook (10, p. 8) came to the same conclusion. Horsfall (9, p. 9), working with aphids, thought that the protein material and calcium pectate were laid down by the plant cells in response to the wound stimulus, although he stated (9, p. 14) that the protein material might possibly have been ejected by the aphid.

Büsgen (1, pp. 43-44), Withycombe (22, p. 70-81), Woods (23, p. 20), and Zweigelt (24, p. 274) concluded that the sheaths were of animal origin. Withycombe and Zweigelt based their conclusions on the fact that they observed the flow of the insect's saliva before or as it made the puncture and this saliva later hardened.

Other observations as to the specific nature of the sheath material are mentioned in the following paragraphs in connection with the substance for which the test was made.

Various workers have employed carbol fuchsin (23, pp. 20-22), safranin (4, pp. 10, 19, 22), and the haematoxylin stains (15) with counter stains for differentiating the sheaths from the plant tissue in histological studies. These stains, although good for permanent mounts, are not satisfactory for quick determinations in fresh material. Several color tests that appear to be desirable differential stains for the sheath material were found during the study reported in this paper.

TECHNIC

Microchemical tests that are described in several available textbooks were used in the present study, and references have been made to these books instead of to the scattered articles originally describing each test. In addition, macrochemical tests were modified for microchemical application. The imperfections of these modifications may account in part for certain inconsistencies in the results, particularly among the tests for the amino acids. Moreover, the negative results obtained with some of the reagents when others used for identification of the same substance were positive may have been due to the insolubility of the sheath in these reagents. Whenever a test was negative, the sheath material was positively identified by testing with Millon's reagent as discussed under the test for tyrosine.

The feeding punctures for study were procured by confining the leaf hoppers in small cages (18) to definite areas on petioles, stems, or leaf veins for periods of 1 or 2 days.⁷ Alfalfa (*Medicago sativa*), apple (*Malus sylvestris*), cowpea (*Vigna sinensis*), Ladino clover

⁷ Fife, in a paper (5) that appeared after the completion of the work reported in the present paper, described a feeding cage for use in studies on *Eutettix tenellus* (Baker) by means of which the insect secretion can be obtained apart from the plant tissue and thus interference by the latter in testing for solubility, crystal character, and optical properties can be eliminated.

(*Trifolium repens latum*), red clover (*T. pratense*) var. Tennessee, sweetclover (*Melilotus officinalis*), and potato (*Solanum tuberosum*) were used as host plants.

The tests were made from 1 to 77 days after the insects had been removed from the plants. The tissue was cut with a razor into slices from 1 to 3 cells thick, and these were placed on slides. The sections containing the natural amber or straw-colored sheaths were separated for study, and the tests observed by means of a microscope. Certain solubility tests and others that were prolonged were carried out in small vials, and the observations were made after the material had been transferred to slides.

RESULTS

The results of the tests for each substance, or group of substances, are presented in tables 1 to 3.

PLANT-RESERVE SUBSTANCES

As table 1 shows, the natural color of the sheath material was not changed by either of the dyes Scharlach R or Sudan III, or by osmic acid. The sheath material did not change when subjected to the color tests for tannin or when allowed to oxidize by exposure on a slide for several days. The negative tests in table 1 indicate the absence of plant-reserve substances in sheaths made by the potato leaf hopper and the alfalfa hopper.

So far as the writer knows, tannin is the only material in this group that has been mentioned by previous investigators in connection with sheath material in plant tissue. Petri (16, p. 28) stated that tannin was deposited about sheaths in feeding punctures by the grape phylloxera. Davidson (4, p. 43) made the same statement with respect to aphids. Painter, working with the chinch bug (15, p. 234), and Withycombe (22, p. 81) with the sugarcane froghopper (*Tomaspis saccharina* Dist.), said that tannins were always present. None of these investigators, however, mentioned the tests they used.

TABLE 1.—Tests for plant-reserve substances in the sheath material surrounding punctures in plant tissue made by *Empoasca fabae* and *Stictocephala festina*

Tested for presence of	Test	Reaction	Reference
Fats and oils	Staining with Sudan III	Negative	Eckerson (pp 12-13) ^a
	Staining with Scharlach R	do.	Do.
	Staining with osmic acid	do.	Chamberlain (3, p. 80)
	Solubility in chloroform	do.	Do
	Solubility in ether	do.	Do
Waxes	Solubility in carbon bisulphide	do.	Do.
	Staining with Sudan III	do	Eckerson (pp 12-13). ^a
Gums, mucilage, gelatinized membranes	Staining with Scharlach R	do	Do.
	Solubility in water	do	Chamberlain (3, p. 82)
Tannins	do	do	Gortner (6, p. 597).
	Reaction with potassium ferricyanide	do	Do.
	Reaction with 10 percent ferric chloride.	do	Eckerson (p. 6). ^a
	Reaction with gold chloride	do	Do. ^a
	Reaction with hydrochloric acid and osmic acid.	do	Do. ^a
	Reaction with ammonium molybdate-ammonium chloride.	do	Withycombe (22)
	Oxidation	do	(Eckerson (p. 6). ^a Gortner (7, p. 598).

^a ECKERSON, S. H. MICROCHEMISTRY. 30 p., n. d. Bot. Dept., Univ. Chicago. [Mimeographed.]

Zweigelt (24, pp. 275-276), using methylene blue as a test, concluded that tannin was present and that the browning of the sheath on exposure was due to oxidation of the tannic acid present. He also stated that in plants poor in tannin there was no accumulation of this material about the sheaths. This observation is in agreement with the results obtained in the present studies and with those of Wells (21, p. 280), who found no tannins in sheaths made by *Pachysylla mamma* Riley and *P. asteriscus* Riley.

CELL-WALL AND WOUND-RESPONSE SUBSTANCES

In the tests for cell-wall and wound-response substances (table 2) the sheath material did not change color when treated with the reagents giving color reactions for lignin and pentosans or with the solvents copper oxide-ammonia, hydrogen peroxide, or potassium chlorate. Nitric acid colored the sheath material a deep yellow. The chronic acid solution dissolved the heavy lignified walls of the xylem vessels and the sheath material in sections at about the same rate, but much more slowly than it dissolved the remaining plant cells. The similar solubility of lignified cell walls and sheath material in the same sections in chronic acid and their insolubility in copper oxide-ammonia do not necessarily indicate similarity between the two substances, however, for negative results were obtained with the other tests and the chitinous insect exoskeleton shows the same reactions.

Horsfall (9, p. 9), using the phloroglucinol test, found no lignin in the sheaths made by aphids.

In the present studies, all tests for callose in the sheath material except the copper oxide-ammonia test were negative. Although the tests employed for its detection are not given, the sheaths made by the grape phylloxera in grape (16, p. 28) and of *Macrosiphum rosarum* Walk. in rose (4, p. 42) are said to contain this substance, and there are indications that it is also present in the sheaths made by *Blissus leucopterus* in corn and sorghum (15, p. 234).

In the tests for cutin and suberin, which were all negative except the test for insolubility in copper oxide-ammonia, the appearance of the sheath material did not change except for the bleaching due to javelle water and Schultze's reagent (ceric acid test). Büsgen (1, p. 44) stated that the sheath was converted to a vacuolated mass in Schultze's reagent, but he attached no significance to the results.

All tests for cellulose in the sheath material were negative. In the presence of concentrated sulphuric acid, iodine-zinc chloride solution, hydrocellulose reaction, iodine, or iodine-potassium iodide this material turned brown. Büsgen (1, p. 44), using the hydrocellulose reaction, obtained a positive test for cellulose in the outer layer of the sheaths made by *Aphis brassicae* L. and *Coccus cacti* L., but Davidson, although he stated (4, pp. 42, 52) that sheaths contained cellulose, obtained negative results with this test and also with copper oxide-ammonia (Schweizer's cupra-ammonia) as a solvent (4, p. 43). Büsgen (1, p. 44) and Prillieux (17, p. 43), using zinc chloridide, obtained negative tests for cellulose in sheaths. Millardet (12, p. 8) mentioned swellings of cellulose following the path of the stylets of the grape phylloxera, but evidently he made no chemical tests.

TABLE 2.—*Tests for plant-cell-wall and wound-response substances in the sheath material surrounding punctures made in plant tissue by Empoasca fabae and Stictopcephala festina*

Tested for presence of—	Test	Reaction	Reference
Lignin	Reaction with phloroglucinol-hydrochloric acid	Negative.	Eckerson (pp. 10, 12) ^a
	Reaction with orcinol-hydrochloric acid	do.	Eckerson (p. 10) ^a
	Reaction with Schultze's maceration fluid followed by the hydrocellulose reaction.	do.	Chamberlain (3, pp. 80-81).
	Insolubility in copper oxide-ammonia	Positive	Eckerson (p. 12) ^a
	Solubility in nitric acid	Negative	Do ^a
	Solubility in hydrogen peroxide	do.	Do ^a
	Solubility in potassium chlorate	do.	Do ^a
Pentosans	Solubility in 50 percent chromic acid	Positive	Eckerson (pp. 12-13) ^a
	Reaction with phloroglucinol-hydrochloric acid	Negative.	Eckerson (p. 10) ^a
	Reaction with orcinol-hydrochloric acid	do.	Do ^a
	Hydrocellulose reaction	do.	Chamberlain (3, p. 81)
Callose	Reaction with zinc chloriodide	do.	Do.
	Insolubility in copper oxide-ammonia	Positive	Eckerson (p. 11) ^a
	Staining with resorcin blue	Negative.	Do ^a
	Staining with aniline blue	do.	Do ^a
	Staining with coralline and sodium bicarbonate.	do.	Chamberlain (3, p. 81).
	Solubility in 1 percent alkali	do.	Eckerson (p. 11) ^a
	Insolubility in copper oxide-ammonia	Positive	Eckerson (p. 13) ^a
Cutin and suberin	Staining with Sudan III	Negative.	Eckerson (p. 12) ^a
	Staining with Scharlach R	do.	Do ^a
	Staining with alcaunin in 50 percent alcohol	do.	Chamberlain (3, p. 82)
	Staining with javelle water and Gruber's cyanin	do.	Do.
	Gerlic acid reaction	do.	Eckerson (p. 13) ^a
	Phellonic acid reaction	do.	Do ^a
	Solubility in 3 percent alcoholic potassium hydroxide	do.	Do ^a
Cellulose and hemi-celluloses	Hydrocellulose reaction	do.	Eckerson (p. 9) ^a
	Reaction with zinc chloriodide	do.	Hawk (8, p. 48).
	Solubility in copper oxide-ammonia	do.	Chamberlain (3, p. 80).
	Solubility in concentrated sulphuric acid	do.	Do
	Staining with iodine solution	do.	Hawk (8, p. 48).
	Staining with iodine-potassium iodide solution	do.	Eckerson (p. 9) ^a
	Reaction with phloroglucinol and hydrochloric acid	do.	Gortner (6, p. 585)
Pectic compounds	Reaction with zinc chloriodide	Positive.	Chamberlain (3, p. 81).
	Solubility in Schultze's reagent	Negative.	Chamberlain (3, pp. 80, 137)
	Solubility in ammonium oxalate	do.	Gortner (6, p. 587)
	Solubility in 2 percent alkalies	do.	Eckerson (p. 12) ^a
	Staining with ruthenium red	Positive.	Eckerson (pp. 11-12) ^a
Starch, amyloid, saponarin, narscine, devil	Staining with methylene blue	do.	Do ^a
	Staining with iodine solution	Negative.	Eckerson (p. 9) ^a
	Staining with iodine-potassium iodide solution.	do.	Do ^a

^a ECKERSON, S. H. (See footnote to table 1.)

In the tests for pectic compounds the sheath material turned brown, as do pectic compounds, in the presence of zinc chloriodide. Ruthenium red and methylene blue stained the sheath material with their respective colors, a positive test for pectic compounds. Eckerson's procedure was then applied to determine to which class of pectic compounds—pectin, pectose, or pectic acid—the staining was due. An acid-soluble substance behaving like pectose was indicated. Horsfall (9, p. 9) and Davidson (4, p. 43) obtained similar results by the same procedure in their studies of sheaths made by aphids, but they called the substance calcium pectate. Withycombe (22, pp. 78-80) obtained positive staining only occasionally with either ruthenium red or methylene blue after treating with hydrochloric acid. Petri (16, p. 28), Wells (21, p. 280), and Painter (15, p. 234) obtained a positive indication of calcium pectate in sheaths, but they did not give the tests they employed.

The sheath material did not appear to be reduced in quantity by the solvents for pectic compounds, and it reacted positively to Millon's reagent after such treatment, indicating that the sheath is composed largely of substances other than pectic compounds. If a pectic compound is present, its solubility indicates a pectoselike form. Tests performed on sheaths produced by leaf hoppers feeding from 1 to 22 days previously indicated that the material does not increase in quantity as time passes. If the material is pectose or some other plant substance and is not produced by the insect, it apparently dissolves and intermixes with the insect's secretion, which retains it on hardening. The absence of pectic compounds is evidenced by the staining of the sheath material on the outside of the epidermis at the point of puncture and also in the intercellular and intracellular spaces within the plant tissue.

PROTEINS AND PROTEINACEOUS SUBSTANCES

Certain color reactions characteristic of either amino acids or peptide linkages were employed in testing for proteinaceous substances (table 3). Some of these reactions are general protein reactions; others are specific for some particular amino acid.

The sheath material became pink to lilac in the biuret test, indicating the presence of derived proteins, such as proteoses or peptones. Büsgen (1, p. 44) obtained a positive biuret reaction in sheaths produced by *Aphis brassicae* and *Coccus cacti*.

A positive xanthoproteic reaction is due to the presence in the protein molecule of benzene nuclei with which the nitric acid forms modifications. The particular complexes of the protein molecule that are of special importance in this connection are those of tyrosine, tryptophane, and phenylalanine. According to Gortner (6, p. 324), the benzene nucleus of phenylalanine is not readily nitrated, and the xanthoproteic reaction is specific for aromatic nuclei that are easily nitrated (tryptophane or tyrosine). Sheath material gave positive color reactions for these two materials but did not dissolve. Hørsfall (9, p. 9) obtained a positive reaction with this test for proteins in sheaths of aphids.

Sheath material, when subjected to the ninhydrin reaction, became a positive greenish-blue color, which indicated the presence of peptones, peptides, or amino acids containing a free carboxyl group and an alpha-amino group.

The negative response to the Molisch reaction indicates that the sheath does not contain a conjugated protein. The negative test with acetic acid-potassium ferrocyanide may have been due to the highly insoluble nature of the sheath material, since the reagent is intended for testing proteins in solution.

Sheath material developed an intense red color⁸ when Sakaguchi's test for arginine, as given by Gortner (6, pp. 325-326), was made. Only compounds containing the free guanidine group react, but this group is free in most naturally occurring proteins. On sheath material the test is very rapid and it is easy to obtain the color contrast between the sheath and the plant parts. A positive test was obtained at room temperature in from 1 to 2 minutes and then the color faded. Gortner

⁸ Since the sheath material was not in solution, 5 cc of water was added to the mixture of sodium hydroxide and alpha-naphthol to make up to the volume of 1 percent protein as recommended.

advises placing the material in the reagent at 2° to 4° C. (35.6° to 39.2° F.) for 40 minutes before examining for color, but slides which the writer prepared and placed on melting ice for 40 minutes showed no color upon examination, the color having faded before the end of this time.

TABLE 3. - *Tests for proteins, amino acids, mucin, and chitin in the sheath material surrounding punctures in plant tissue made by Empoasca fabae and Sticlocephala festina*

Tested for presence of—	Test	Reaction	Reference
General proteins.	Buret reaction.....	Positive.	Gortner (6, p. 323).
	Ninhydrin reaction.....	do.....	Do
	Xanthoproteic reaction.....	do.....	Mathews (11, pp 154-155)
	Molisch reaction.....	Negative.	Gortner (6, p. 325).
	Reaction with acetic acid-potassium ferrocyanide.....	do.....	Hawk (8, p 103).
Arginine....	Reaction with Sakaguchi's test.....	Positive	Gortner (6, pp 325-326)
Asparagine and glutamine	Reaction with quinone solution.....	do.....	Eckerson (p. 19) ^a
	Reaction with copper acetate and alcohol.....	Negative.	Do ¹
	Reaction with Ehrlich's reagent.....	do	Gortner (6, p. 325)
Tryptophane	Reaction with Acree-Rosenheim test.....	do	Do
	Reaction with Liebermann's test.....	Positive	Gortner (6, p 324).
	Reaction with Adamkiewicz test.....	do.	Gortner (6, p. 325).
	Benedict's modification of Hopkins-Cole reaction.....	do.....	(Hawk (8, p 98).
	Reaction with Folin and Denis' reagent.....	Negative	Mathews (11, p 981).
Tyrosine	Reaction with Millon's reagent.....	Positive.	Hawk (8, p 86)
	Reaction with Folin and Denis' reagent.....	do.....	(Eckerson (p 19) ^a
	Reaction with Morner's reagent formaldehyde-sulphuric acid.....	Negative	Mathews (11, pp 980 981).
	Reaction with sodium molybdate-sulphuric acid test.....	do.....	Hawk (8, p. 86).
	Staining with thionine blue.....	do	Morrow (13, p 117)
Mucin.	Reaction with alkali and Ehrlich's reagent.....	do	Eckerson (p 19) ^a
	Reaction with ferric chloride and hydrochloric acid.....	do	Mathews (11, p 335).
	Solubility in concentrated hydrochloric acid.....	do	Mathews (11, p 337)
	Solubility in concentrated potassium hydroxide.....	do	Hawk (8, p. 85).
	Solubility in 0.5 percent sodium hydroxide.....	do	(^b)
Chitin	Solubility in 50 percent chromic acid.....	do.....	(^b).
	Chitosan reaction.....	Positive	(^b)
			Eckerson (pp 12-13). ^a
			(Eckerson (p 13). ^a
			(Campbell (2, pp. 404-405).

^a ECKERSON, S. H. (See footnote to table 1.)

^b In tests made by the author, mucin, precipitated from saliva, dissolved in these reagents within 48 hours but sheath material did not.

Sheath material gave a cherry-red color reaction after about 15 minutes in the cold in the presence of quinone solution, indicating the presence of asparagine or glutamine. Chitinous walls of leaf-hopper legs did not give the color reaction, but the muscles and apodemes did. Other tests for differentiation of these amino acids as given by Eckerson ⁹ were negative. The highly insoluble nature of the sheath material probably prevented any of its components from going into solution and thus accounted for the negative results.

Three of the tests for tryptophane in sheath material were positive, i.e., Liebermann's, Adamkiewicz, and Benedict's modification of the Hopkins-Cole reaction. Benedict's modification of the Hopkins-Cole test produced a reddish-violet coloration of sheath material in plant sections after 10 to 15 minutes in the cold. This test seems to be excellent for color differentiation, since the sheath was the only

⁹ ECKERSON, S. H. (See footnote to table 1.)

material in the plant section that took the color and the results were obtained quickly and without distortion of cell structure.

Although the Acree-Rosenheim test for tryptophane gave no color reaction when applied to sheaths or chitinous body walls of leaf hoppers, the vascular tissues in sections from badly injured stems gave positive violet to black colorations, in contrast to uninjured tissue. This indicates that proteins may accumulate, as well as carbohydrates, as previously indicated (7), owing to interference in translocation by the feeding of the insects.

In the tests for tyrosine the sheath material became brick red when treated with Millon's reagent. The test is quickly made and the contrast makes the reagent a useful one in detecting feeding punctures.

Büsgen (1, p. 44) was the first to report a positive test on sheath material with Millon's reagent and commented on its specific color reaction. Morstatt (14, pp. 354-355) was unable to obtain a positive color test with this reagent on sheaths of the scale insect *Diaspis fallax* Horvath. Zweigelt (24, p. 275) believed that the positive test obtained with Millon's reagent did not mean that no other substances were included, and Petri (16, p. 9, footnote) believed that the test indicated an albumin-tannic acid combination. In some of the sheaths of *Stictoccephala festina* the tubular wall seemed to consist of two layers, an inner, dark brick-red, thin-walled layer of rather definite outline and an outer, lighter red accumulation less regular in outline. Painter (15, p. 234) observed the two layers in the sheath of the chinch bug. The first evidence of the plugging of xylem vessels by sheath material was obtained by the writer (19, p. 278) with this reagent. In tests with Millon's reagent on the chitinous parts of the bodies of *Empoasca*, a similar red color was observed in the veins of the wings and in the spines and walls of the legs. The similarity in the response of the insect exoskeleton and the sheath material secreted by the same insect is extremely interesting. This reaction is given by all organic compounds containing a monohydroxybenzene nucleus, including phenol and salicylic acid (11, p. 154). According to Gortner (6, p. 324), the red coloration is specific for tyrosine in protein substances.

Sheath material and chitinous insect parts gave negative tests for tyrosine when treated with Folin and Denis' reagent, Mörner's reagent, or sodium molybdate-sulphuric acid. These materials became chestnut brown in the presence of the last two reagents. The vascular tissue in the badly injured sections gave positive blue color reactions when treated with Folin and Denis' reagent or with sodium molybdate-sulphuric acid. The greater brilliance of the blue color observed in treated sections of injured plants as compared with that seen in uninjured tissue of the same age suggests the possibility of local accumulation of proteins in addition to starch (?) as a result of insect feeding and the consequent interference with translocation of plant material.

In the tests for mucin in the sheath material, chitinous parts of leaf hoppers and chitosan produced from chitin gave negative reactions when treated with Ehrlich's reagent, ferric chloride and hydrochloric acid, or with thionine blue, as compared with mucin from saliva. These materials did not dissolve in concentrated hydrochloric acid, potassium hydroxide, or in 0.5 percent sodium carbonate, as did mucin.

Horsfall's test with thionine blue for mucin in sheaths of aphids was negative (9, p. 9).

Büsgen (1, p. 44) and Davidson (4, p. 43) stated that javelle water dissolved the sheaths of aphids. In the present study sheath material was dissolved in 1½ hours by several changes of javelle water.

Chitin is relatively insoluble in 50 percent chromic acid. In tests with this reagent on sheaths in plant sections, as indicated under the tests for lignin, all the cells except traces of the xylem tubes dissolved at the end of 22 minutes, but portions of the sheath walls remained. Chitinous walls on legs of leaf hoppers were apparently unchanged by a like treatment, but these parts are much larger than the thin walls of the sheath.

Sheath material, when tested for chitosan, gave a positive delicate violet color. Three percent acetic acid dissolved the sheath material after it had been changed by the chitosan procedure. Upon addition of 75 percent sulphuric acid the violet-colored sheath dissolved as the color faded; then, after a short interval, the cellulose walls of the plant cells exhibited the typical hydrocellulose reaction. With the exception of the test for chitosan sulphate crystals, the chitosan procedure was checked by carrying out the tests upon chitinous body parts of *Empoasca*. A flocculent material in the cells, particularly of the vascular bundle, gave a blue color with iodine-potassium iodide and often interfered with the observations on the sheaths. This was avoided by boiling the sections in 12 percent hydrochloric acid for 10 minutes before heating them in potassium hydroxide. The quantity of material that gave the test for chitosan was much less than that which gave a positive test with Millon's reagent.

These tests indicate that chitin is present in the sheaths of these insects, and, so far as the writer is aware, constitute the first evidence to be reported that this substance is secreted by the salivary glands of an insect.

SUMMARY AND CONCLUSIONS

A study has been made of the chemical nature of the sheaths found in feeding punctures produced by the potato leaf hopper (*Empoasca fabae*) and the three-cornered alfalfa hopper (*Stictiocephala festina*). Tests were made for materials that might be derived from the surrounding plant cells and for materials that might be produced by the insects. The results are compared with those of previous workers.

In all tests, the sheaths made by the two species responded similarly.

All tests for plant-reserve, wound-response, and plant-cell-wall substances except the color tests for pectin compounds were negative.

Color tests for mucin were negative, but certain color tests for proteins and amino acids showed that the sheath material is largely proteinaceous.

The sheath material may contain chitin, as it responded similarly to chitinous exoskeleton in a number of tests.

In the course of this study four tests were found which differentiate sheath material from plant tissue by color, namely, Adamkiewicz, Sakaguchi, quinone solution, and Benedict's modification of the Hopkins-Cole reaction.

It appears that the sheath is largely of insect origin and contains no plant substance, with the possible exception of pectose. The highly insoluble sheath, persisting in the vascular tissue, probably interferes

with the normal translocation of plant materials and accounts for the typical plant injuries caused by the feeding of *Empoasca fabae* and *Stictocephala festina*.

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VITAMIN A CONTENT OF BARLEY¹

By E. H. HUGHES

Associate animal husbandman, California Agricultural Experiment Station

INTRODUCTION

It has been shown by Steenbock, Kent, and Gross² that barley, when fed alone, is unable to meet the needs of the young, growing animal. These workers have demonstrated that barley is deficient in inorganic salts and in the fat-soluble vitamins.

Barley, in the great valleys of California, is grown at a lower altitude and under very different climatic conditions than is the case in the Corn Belt. If barley, already deficient in fat-soluble vitamins, particularly vitamin A, be exposed to the extreme temperature of harvest time in California, the content of this vitamin may be still further depressed.

EXPERIMENTAL PROCEDURE AND DATA

To test the vitamin A content of barley and to compare it with that of yellow corn and white corn, three experiments were conducted by the writer. The rat was used as the experimental animal. Results in the first experiment were determined by increased growth as measured by body weight, the development of eye lesions, and other external evidences of vitamin A deficiency. The additional measure of the effects of these diets on the oestrous cycle of the rat was included in the second experiment. Evans and Bishop³ have shown that the constant appearance of cornified cells in the vaginal smear is a characteristic test for a deficiency of the fat-soluble vitamin A in the diet. A quantitative determination of the vitamin A content of barley compared with that of yellow and white corn, after the method of Smith,⁴ was the object of the third experiment.

EXPERIMENT 1

In the first experiment the vitamin A content of barley was compared with that of yellow corn and white corn, because in 1919 Steenbock⁵ demonstrated that white corn had very little vitamin A and that yellow corn had sufficient vitamin A for growth and reproduction in the rat.

Stock male rats were used and were fed the diets shown in table 1.

The rats were kept in steel cages 15 inches long, 10 inches wide, and 11 inches high, not more than five rats being in each cage. The cages were equipped with a raised wire-mesh bottom. The mesh was of a

¹ Received for publication Apr. 10, 1933; issued October, 1933.

² STEENBOCK, H., KENT, H. E., and GROSS, E. G. THE DIETARY QUALITIES OF BARLEY. *Jour. Biol. Chem.* 35: 61-74, illus. 1918.

³ EVANS, H. M., and BISHOP, K. S. ON AN INVARIABLE AND CHARACTERISTIC DISTURBANCE OF REPRODUCTIVE FUNCTION IN ANIMALS REARED ON A DIET POOR IN FAT SOLUBLE VITAMIN A. (Abstract) *Anat. Rec.* 23: 17-18. 1922.

⁴ SMITH, M. C. A QUANTITATIVE COMPARISON OF THE VITAMIN-A CONTENT OF YELLOW CORN AND THE GRAIN SORGHUM HEGARI AND YELLOW MILO. *Jour. Agr. Research* 40: 1147-1153, illus. 1930.

⁵ STEENBOCK, H. WHITE CORN VS. YELLOW CORN AND A PROBABLE RELATION BETWEEN THE FAT-SOLUBLE VITAMIN AND YELLOW PLANT PIGMENTS. *Science (n.s.)* 50: 352-353. 1919.

sufficient size to allow the feces to drop through out of reach of the animals. A supply of distilled water and feed was kept in the cages at all times during the experiment.

TABLE 1.—*Constitution of diets used in experiment 1*

Vitamin-A-free diet		McCullum's diet 1		Diet A		Diet B		Diet C	
Constituent	Percent	Constituent	Percent	Constituent	Percent	Constituent	Percent	Constituent	Percent
Dextrin	70	Whole wheat	67.5	Yellow corn.....	92	White corn.....	92	Barley.....	92
Casein (extracted)	18	Casein (extracted)	15.0	Casein (extracted)	6	Casein (extracted)	6	Casein (extracted)	6
Dried yeast	6	Whole milk powder.....	10.0	CaCO ₃	1	CaCO ₃	1	CaCO ₃	1
Agar.....	2	CaCO ₃	1.5	NaCl.....	1	NaCl.....	1	NaCl.....	1
Salt mixture (Steenbock 40)	4	NaCl.....	.8						
		Butter.....	5.2						

Unextracted casein was used for a few days at the beginning of the experiment before extracted casein was available. The casein was extracted with fast-boiling 95 percent alcohol, air being bubbled through for a considerable part of the time. It was dried in the sun for 15 hours.

The barley, yellow corn, and white corn used were recleaned to insure freedom from weed seeds and other foreign matter and were finely ground in an experimental mill of the hammer type.

The group receiving the vitamin-A-free diet was used as a check to be sure that the extracted casein did not contain vitamin A. The animals on this diet gained in weight for a time, then lost weight rapidly. After losing considerable weight, four were given 2 drops of cod-liver oil daily and made rapid recovery, two were not given cod-liver oil daily and both died. They showed evidence of a vitamin A deficiency.

Growth curves of animals fed the stock diet (McCullum's diet 1) are represented in figure 1, *A*. These experimental animals showed normal growth as compared with other males of the colony.

The yellow-corn diet proved not so efficient for growth (fig. 1, *B*) as did the normal stock diet (fig. 1, *A*). There was no evidence of vitamin A deficiency manifested by any of the animals; while growth was not normal on this diet when compared to those fed McCullum's diet 1, the individuals, as measured by gain in weight, were uniform in their increase.

Rats fed the white-corn diet gained very slowly, and all showed evidences of a deficiency of vitamin A. Animals 563 and 569 were given cod-liver oil, one at the end of 10 weeks, the other at the end of 12 weeks. Both recovered rapidly, as indicated by growth curves (fig. 1, *C*). All other animals fed this diet died except rat 649. This rat never weighed more than 120 g but was still alive at the end of the eighteenth week.

An examination of growth curves (fig. 1, *D*) of animals fed a barley diet indicates a lack of growth as compared to those fed yellow corn (fig. 1, *B*). This was particularly true of animals put on this diet at a weight of 40 g. None of them made as rapid or as consistent growth as those fed the yellow-corn diet. However, rats 632 and 637

were heavier when they were put on the barley diet, and finally attained a weight of 220 g. An improvement in growth was manifested by rat 568 when from 2 to 3 drops of cod-liver oil was given, after it had shown a gradual decrease in weight for 5 weeks. Some improvement was shown by no. 0 and no. 625. Rat 625 was given yeast, the

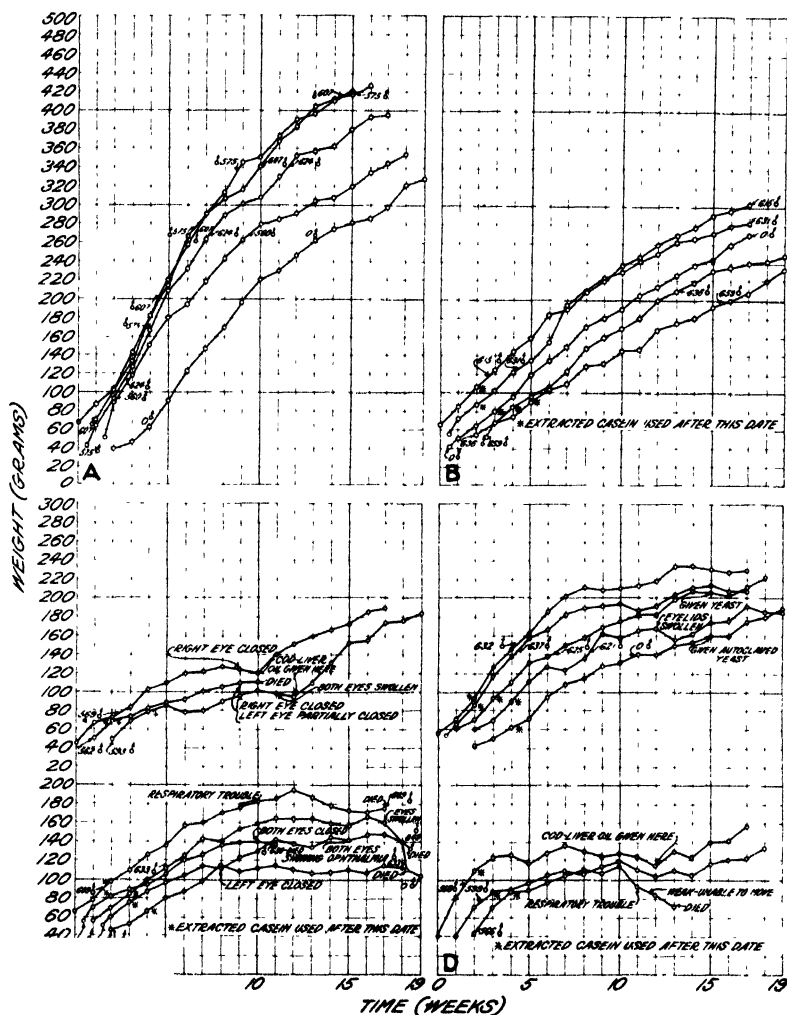


FIGURE 1.—*A*, growth of male rats fed McCollum's diet 1; *B*, growth of male rats fed yellow corn as their sole source of vitamin A; *C*, growth of male rats fed white corn as their sole source of vitamin A, with and without the addition of cod-liver oil; *D*, growth of male rats fed barley as their sole source of vitamin A, with and without the addition of cod-liver oil or yeast.

other autoclaved yeast. No serious ophthalmic disturbances were noticed in those individuals on a barley diet.

When growth is used as a measure of the vitamin A content of yellow corn, white corn, and barley, the indications are that the vitamin A content of barley lies somewhere between that of yellow corn and white corn.

EXPERIMENT 2

In experiment 2, the vitamin A potency of barley as it relates to that of yellow corn and white corn was tested (1) by growth as measured by body weight, and (2) the occurrence of the oestrous cycle as determined by daily vaginal smears. Female rats were used in this experiment.

Evans⁶ has shown that a deficiency of vitamin A in the diet results in the constant appearance of cornified cells in the vaginal smear. This is an earlier test of deficiency and probably is more constant in its appearance than are other tests for a lack of this vitamin.

Figures 2, *A* and 2, *B*, represent a complete record of the vaginal smears of rats on the three diets. An additional record of the smears of three individuals from the station normal colony is also shown.

The dashes indicate a period of time in which no smear records were taken. The time in days represents the period in which smears were taken after the opening of the vagina.

Individuals on McCollum's diet 1 showed a regular and normal oestrous cycle.

The vaginae of the animals fed the white-corn diet never opened; therefore, there could be no smear records for them (fig. 2, *A*). The vaginae of rats B1109, W1113, and W1112, fed a barley diet, opened when the rats were about 70 days of age, which was much later than for those fed McCollum's diet 1. Soon after the opening of the vaginae, these rats cycled, W1112 for a period of about 20 days, the other two for a much shorter period. They then showed the cornified cells constantly, until they were fed, for 1 day, a mixture of diets A and B (yellow and white corn); then they began cycling and continued to cycle for a few days. A mixture of equal parts by weight of diets A and B apparently contained a higher potency of vitamin A than did the barley diet. Rat B1109 then showed a constant smear of cornified cells until death. On the one hundred and fifteenth day, 1 percent wheat-germ oil was added to the diet of rat W1113, and in a few days leucocytes and epithelial cells appeared with the cornified. Not until cod-liver oil was added on the one hundred and fifty-second day was there a loss of the cornified cells in the daily smears.

Two drops of cod-liver oil were given to rat W1112 for 14 days, beginning on the seventy-fifth day. This individual began to cycle and continued until the end of the experiment. The addition of 1 percent of wheat-germ oil on the one hundred and twenty-fifth day seemed to depress the cycle. This individual did not cycle so continuously as before, until cod-liver oil was again added on the one hundred and sixtieth day.

Vaginal-smear records of other rats on a barley diet and of three on a yellow-corn diet are presented in figure 2, *B*. Individuals W1103 and W1106 (yellow-corn diet) were very regular and uniform in their cycling. W1110 was not so regular, particularly after the one hundred and eighty-fifth day. The records of the rats on the barley diet are very similar to those shown in figure 2, *A*. Each began cycling after a day's feeding of diets A and B mixed. This was followed by a constant cornified-cell smear until either cod-liver oil or wheat-germ oil was added. Rat G1096 received cod-liver oil

⁶ EVANS, H. M. THE EFFECTS OF INADEQUATE VITAMIN A ON THE SEXUAL PHYSIOLOGY OF THE FEMALE. *Jour. Biol. Chem.* 77:651-654. 1928.

on the forty-fourth day and began cycling on the forty-sixth day and continued to cycle until the end of the experiment. Rats W1108 and B1100 dropped to dioestrus immediately upon the addition of

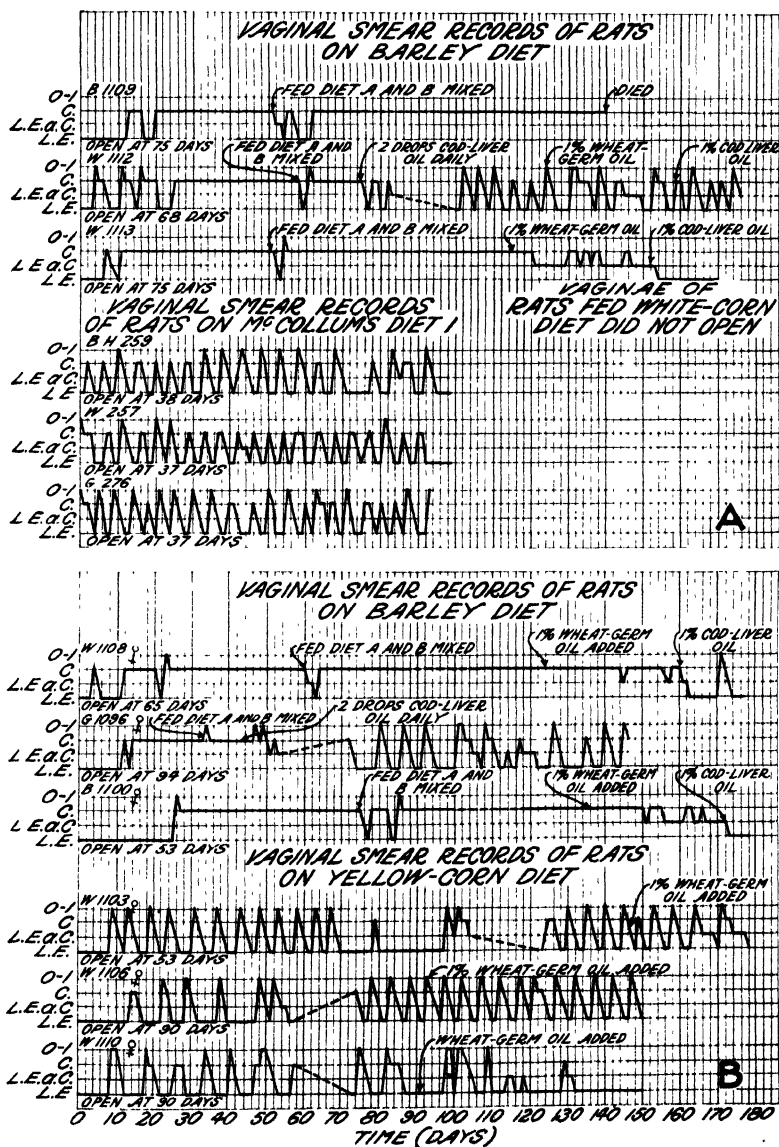


FIGURE 2.—A, vaginal-smear records of female rats fed barley as their sole source of vitamin A, as contrasted with records of female rats fed McCollum's diet 1; B, vaginal-smear records of female rats fed barley and yellow corn as their sole source of vitamin A. (O-1 represents epithelial and cornified cells; C represents cornified cells; L. E. a. c. represents leucocytes, epithelial, and cornified cells; and L. F. represents leucocytes and epithelial cells.)

cod-liver oil toward the end of the experiment. Rat W1108 showed one apparently normal cycle just before the conclusion of the experiment. The addition of wheat-germ oil to the extent of 1 percent did

not result in a loss of cornified cells in the smears of rats E1106 and B1100. When growth and vaginal smears are used as a measure of the vitamin A content of barley, it is shown that the quantity in barley is low; in fact, it is apparently much lower than that of yellow corn but is higher than that of white corn.

EXPERIMENT 3

The results of the studies (experiments 1 and 2) indicate that barley is low in its vitamin A content and that its potency lies somewhere between that of yellow and of white corn. Further studies to compare quantitatively the amount of this vitamin in barley and yellow and white corn are reported in experiment 3. The technic and criteria used were those of Smith,⁷ being a method developed by Sherman and Munsell.⁸

The yellow corn used was a good grade (at least no. 2) of yellow dent corn purchased locally. White corn of excellent quality (offered

for sale as seed corn) was purchased from a seed store. The variety was unknown. Locally grown Yolo County barley of good quality, free from foreign matter and weed seeds, was used.

Normal female and male rats from the station experimental colony were used in this series. The females were bred, and during the gestation and lactation periods they were fed Sherman's diet B, except that green alfalfa was given occasionally instead of lettuce. This diet was composed of two thirds ground wheat, one third whole-milk powder, and sodium chloride equal to 2 percent of the wheat.

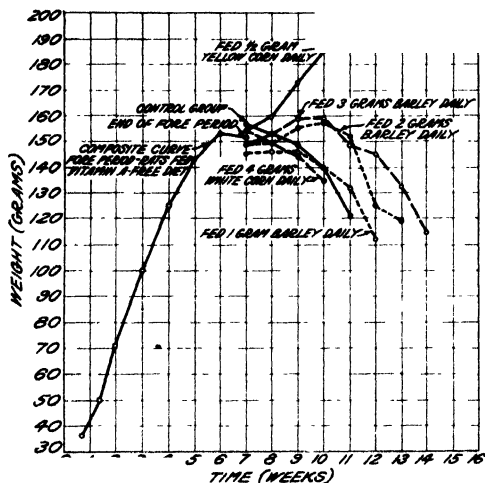


FIGURE 3.—Growth records of male and female rats fed a vitamin-A-free diet until the end of the seventh week, designated as the fore period, and thereafter fed the indicated quantities of barley, white corn, and yellow corn daily.

The young rats from these mothers were weaned at from 20 to 25 days of age and weighed an average of about 37 g each. They were then placed on a vitamin-A-free diet composed of 67 percent of corn-starch, 18 percent of casein (rendered vitamin-A-free by successive extractions with boiling 95 percent alcohol), 10 percent of dried brewer's yeast, 4 percent of salt mixture (Osborne and Mendel's), and 1 percent of sodium chloride. The vitamin D was incorporated by irradiating the dried brewer's yeast, spread out in thin layers, in the direct rays of the sun for 1 hour and 20 minutes.

From weaning time on these young rats were kept in metal cages with screen bottoms. At the end of the fourth week they began showing the effects of the vitamin-A-free diet. A week later some of them were losing weight and showing typical outward symptoms of vitamin A deficiency. Figure 3 shows graphically these weight

⁷ SMITH, M. C. Op. cit.

⁸ SHERMAN, H. C., and MUNSELL, H. E. THE QUANTITATIVE DETERMINATION OF VITAMIN A. *Jour Amer. Chem. Soc.* 47: 1639-1646, illus. 1925.

changes. At the end of the sixth week all had lost weight, or their weight had remained stationary for at least a week.

Each rat was then transferred to a separate cage and fed individually until death, or until the end of the feeding period. Water was provided at all times to each animal. The vitamin-A-free diet was fed in cups and was always available to the individual. The yellow corn, white corn, and barley were weighed and fed daily in small glass dishes. These cereals were very finely ground in an experimental mill of the hammer type.

The 6 groups of 6 rats each were fed the following diets:

- Group 1, vitamin-A-free diet (control group)
- Group 2, vitamin-A-free diet and $\frac{1}{2}$ g yellow corn daily
- Group 3, vitamin-A-free diet and 4 g white corn daily
- Group 4, vitamin-A-free diet and 1 g barley daily
- Group 5, vitamin-A-free diet and 2 g barley daily
- Group 6, vitamin-A-free diet and 3 g barley daily

TABLE 2.—Average gain or loss in weight and average survival period of animals in experiment 3

[6 rats in each group]					
Group no. ^a	Average initial weight	Average weight, end of fore period	Average first appearance of ophthalmia in rats	Average gain or loss in weight in 56 days	Average survival period
	Grams	Grams	Days	Grams	Days
1	37.7	157.0	28.3	-56.3	8.2
2	37.3	153.8	^b 56.0	+43.3	---
3	39.3	153.8	35.5	-29.3	13.2
4	36.3	143.2	37.0	-36.8	17.0
5	38.8	148.3	44.3	-50.0	24.8
6	35.8	148.8	48.2	-39.8	23.0

^a See fig. 3 and text for diet fed the animals in the various groups

^b They were actually carried 68 days before being taken off the diet

In table 2 and figure 3 are presented the findings in this study. All the animals in group 2 survived and showed no evidence of vitamin A deficiency at any time during the 56 days. Only two other individuals survived. One rat in group 4 died on the fifty-sixth day, and one individual in group 6 lived 67 days after the end of the fore period.

The rats in group 2 were the only ones that showed an average constant gain in weight. An average gain of 43.3 g each for individuals in group 2 indicates that 0.5 g of yellow corn contains more than 1 unit of vitamin A, which is in agreement with the results of Smith.⁹ The animals in group 1 (control group), and in group 3, which were fed 4 g of white corn daily, continued to lose weight after the end of the fore period and survived an average of only 8.2 and 13.2 days, respectively. Slight gains were manifested by the experimental animals in groups 4, 5, and 6 for a short period of time after barley was added to the vitamin-A-free diet. Group 4, fed 1 g of barley daily, gained very slightly for 2 weeks, then lost weight rapidly. Groups 5 and 6 gained in weight for 4 weeks, then lost weight rapidly. Group 6, receiving 3 g of barley, gained slightly more than did group 5, fed 2 g of barley daily. None of the rats in groups 4, 5, or 6 survived the 8-week period.

⁹ SMITH, M. C. *Op. cit.*

Three grams of barley fed daily to each individual as the only source of vitamin A was not sufficient to enable the animals to survive the 56-day feeding period. The individuals that received 2 and 3 g of barley each survived somewhat longer than those that received only 1 g of barley daily. All individuals except those fed yellow corn developed ophthalmia at some time before death. Some developed respiratory difficulties. The first appearance of ophthalmia varied somewhat in the various groups; those receiving the 2 and 3 g of barley daily did not show this condition as early as did those fed white corn or 1 g of barley.

All the evidence indicates that barley is low in its vitamin A content when compared with yellow corn. It apparently contains more vitamin A than does white corn. From the facts set forth in figure 3 and table 2 the barley used in this study, which is believed to be an average California barley contained less than one sixth as much vitamin A as did the yellow corn.

SUMMARY

Barley as the only source of vitamin A in the diet did not produce normal growth in rats.

In the sexually immature rat the addition of vitamin A to a barley diet (barley being the only source of vitamin A) resulted in a decided increase in growth and a normal oestrous cycle.

As measured by increase in growth and by daily vaginal-smear record of rats, barley contains more vitamin A than does white corn, but less than yellow corn.

In these studies it has been shown that barley contains less than one sixth as much vitamin A as does yellow corn.

ASSIMILATION OF FIXED NITROGEN BY GRASSES AND CLOVERS¹

By A. B. BEAUMONT, *head, Department of Agronomy*, W. S. EISENMENGER, *research professor of agronomy*, and W. J. MOORE, JR., *assistant in agronomy*, Massachusetts Agricultural Experiment Station²

INTRODUCTION

In the research herein reported certain chemical forms of nitrogen commonly present in commercial fertilizers have been used—nitric, ammoniacal, and amide forms— but no attempt was made to use all the different compounds carrying one or another of these forms of nitrogen. The purpose of these studies was to determine the readiness with which these common forms of nitrogen are absorbed and assimilated by the grasses and clovers usually grown for forage. Methods which involved the growing of plants in nutrient solutions seemed most suitable for the ends sought and were therefore used. Both unsterilized and sterilized culture solutions were used. Each of these methods of growing plants has certain advantages and disadvantages over the other, as will appear later.

METHOD USED WITH UNSTERILIZED CULTURES

Pure seedlings of the grasses and clovers used in the experiments were grown in flats. The stands were thick, and on account of the crowded condition of the seedlings and the limited supply of available nutrients (conditions desired in this case), the growth was slow. When about 5 months old, seedlings were transplanted to water cultures, care being exercised to remove as much of the adhering soil as possible. Six seedlings of each grass species, except orchard grass, and four seedlings of orchard grass and each clover species, comprised the individual cultures. There were eight cultures of each treatment. The seedlings were supported by a cork with one hole in it. It was found that plants would remain in place, after some growth, without the aid of cotton which has some disadvantages in such work. Glass bottles of 8-ounce capacity were used. Other details of equipment were similar to those shown in figure 1 of a previous paper (3).³

A standard nutrient solution containing all nutrients supplied except the nitrogen, and of the following composition was used:

	<i>Mol</i>
CaHPO ₄ · 2H ₂ O.....	0. 00055
CaSO ₄ · 2H ₂ O.....	. 00130
MgSO ₄ · 7H ₂ O.....	. 00285
KCl.....	. 00188

¹ Received for publication May 22, 1933; issued October, 1933. Published as contribution no. 163 of the Massachusetts Agricultural Experiment Station.

² Credit is given G. Rohde and H. Papendieck, formerly assistants in agronomy, for assistance with the solution cultures.

³ Reference is made by number (*italic*) to Literature Cited, p. 503.

To this were added 10 parts per million of ferric potassium tartrate, 3 parts per million of boric acid, and the specific nitrogen carrier under consideration. The concentration of sodium nitrate was 0.0139 mol, and ammonium sulphate and urea were used in concentrations to furnish a chemically equivalent amount of nitrogen. The reaction of the fresh nutrient solution was in all cases approximately pH 7.0.

Solutions containing minerals only, sodium nitrate and ammonium sulphate, were changed weekly. This frequency was sufficient to prevent the formation, in detectable quantities, of nitrates in the ammonium sulphate solution. Cultures containing urea (series 1) were changed every 3 days. In the course of the experiment it was found that ammonia was formed from the urea. Therefore, a new urea series (2) was set up with a more frequent change of solution. The schedule finally adopted for this series was that of keeping the plants in the solution for 1 day only, changing them to water containing no added salts for 1 day, and then on the third day transferring them to a new nutrient solution. In this way ammonification of urea was either prevented entirely or was reduced to a minimum. Ammonia was occasionally detected in some of the cultures.

There was some variation in the length of the period the plants were grown in the nutrient solutions. The duration of the period was 49 to 50 days for solutions containing minerals only and sodium nitrate; for series 1 urea, the period was 43 days for the grasses, and 49 for clovers; for urea 2, 48 days; for ammonium sulphate, 40 days; and for ammonium sulphate plus calcium carbonate, 37 days. In cases in which the period was less than 48 days the plants were harvested because they had apparently made their maximum growth and were beginning to deteriorate. Dry weights of tops and roots are given in table 1.

TABLE 1.—Mean dry weight (grams) of various grasses and clovers when grown in different nutrient solutions

Crop	Mean dry weight of tops						Mean dry weight of roots					
	Check	Sodium nitrate	Ammonium sulphate	Ammonium sulphate plus calcium carbonate	Urea 1	Urea 2	Check	Sodium nitrate	Ammonium sulphate	Ammonium sulphate plus calcium carbonate	Urea 1	Urea 2
Grasses.												
Timothy...	0.500	2.51	0.615	0.904	1.97	1.87	0.400	0.364	0.158	0.151	0.210	0.213
Redtop	.323	2.73	.710	1.085	1.31	2.32	.355	.431	.113	.140	.094	.180
Orchard	.462	2.07	.749	.650	1.68	2.03	.351	.240	.096	.095	.276	.246
English ryegrass ..	.746	2.74	.641	1.046	2.37	2.28	.678	.536	.121	.159	.406	.289
Kentucky bluegrass..	.326	1.09	.471	.706	.859	1.41	.253	.270	.053	.108	.118	.339
Mean	.471	2.23	.637	.880	1.64	1.98	.407	.368	.108	.131	.221	.253
Clovers:												
Red	1.92	1.44	.396	.943	2.07	---	.458	.340	.105	.216	.516	---
Alsike	1.48	1.55	.241	.694	1.81	3.00	.431	.298	.061	.114	.339	.675
White	.725	1.75	.314	.565	1.85	2.55	.170	.286	.001	.120	.294	.654
Mean	1.38	1.58	.317	.734	1.91	2.78	.353	.308	.076	.150	.383	.665

* Only 4 plants grown in each culture. Figures reported are corrected to make results comparable with other grasses, of which 6 plants were grown.

The grass species grown were timothy (*Phleum pratense*), redtop (*Agrostis alba*), Kentucky bluegrass (*Poa pratensis*), orchard grass (*Dactylis glomerata*), English ryegrass (*Lolium perenne*).

The clover species were red (*Trifolium pratense*), Alsike (*T. hybridum*), and white (*T. repens*).

CHEMICAL ANALYSIS OF THE PLANTS

In order to obtain some information regarding changes undergone by the absorbed nitrogen, determinations of total, water-soluble, ammoniacal, and nitric nitrogen were made. It was necessary to combine all the grass species and all the clover species in order to obtain enough material for analysis. This procedure appeared justifiable on account of the marked similarity in the growth behavior of the grasses on the one hand and the clovers on the other. However, it should be noted that some differences in the reaction of the species were observed.

In plant material containing no nitrates, total nitrogen was determined by the official Gunning method (1). In determining the water-soluble nitrogen 1.5 g of dry ground material was shaken with 150 cc of water for 1 hour, and 50 cc of the filtrate used for analysis. This was evaporated to dryness on a water bath and the total nitrogen determined by the Gunning method. Ammonia was determined by distillation of 1 g of plant material in 500 cc of water containing 5 g of magnesium oxide, according to the official method (1).

In plant material containing nitrates the total nitrogen was determined by the modified method of Ranker (7), which involves the reduction of nitrates with sodium thiosulphate. Water-soluble nitrogen was extracted as described above, and then the method described by Pucher and others (6) was used. Ammonia was determined by the method described above. Nitrates were determined by the method suggested by Vickery and Pucher (8).

Analytical data are given in table 2.

TABLE 2. - *Total and fractional nitrogen content (in percentage of dry weight) of grass and clover cultures grown in different nutrient solutions*

Crop and nitrogen carrier	Tops				Roots			
	Total nitrogen	Water-soluble nitrogen	Ammoniacal nitrogen	Nitric nitrogen	Total nitrogen	Water-soluble nitrogen	Ammoniacal nitrogen	Nitric nitrogen
Grasses:								
Check.....	0.96	0.28	0.05	0	0.76	0.14	0.13	0
Sodium nitrate.....	2.97	1.02	.03	.30	2.37	.57	.02	.27
Ammonium sulphate.....	4.19	2.33	1.33	0	2.63	1.11	.56	0
Ammonium sulphate plus calcium carbonate.....	4.03	2.16	1.28	0	2.38	.40	.25	0
Urea 1.....	3.72	1.75	.19	0	2.73	.75	.16	0
Urea 2.....	2.81	1.19	.13	0	2.46	.67	.16	0
Clovers:								
Check.....	2.81	.74	.12	0	3.34	.97	.25	0
Sodium nitrate.....	3.04	1.24	.05	.33	2.89	.94	.07	.41
Ammonium sulphate.....	3.61	1.74	.75	0	4.25	1.49	.56	0
Ammonium sulphate plus calcium carbonate.....	4.02	1.90	.55	0	4.06	1.65	.33	0
Urea 1.....	3.39	1.41	.13	0	4.34	1.76	.21	0
Urea 2.....	3.53	1.49	.09	0	4.00	1.58	.24	0

REACTION OF THE NUTRIENT SOLUTIONS

The reaction of the nutrient solution before it was used, and without the addition of calcium carbonate, was pH 7.0. The reaction of each treatment was determined colorimetrically at the time of changing solutions; that is, after each growth interval. The data are given in table 3. For solutions carrying ammonium sulphate without calcium carbonate, two pH values are given. The first value represents the end point in the first stages of the experiment when plant growth was vigorous, and the second the end point near the close of the experiment when the plant had practically ceased to function. Grasses which were less susceptible to the conditions present in, or caused by, the solution, both effected a lower pH value and held it lower than did the clovers. The data show interesting relationships for the other solutions. In all the remaining solutions, except ammonium sulphate plus calcium carbonate, the pH values were generally lower for the clovers than for the grasses at the close of each interval. This would indicate the normal physiological effects to be expected. For example, the clover roots were well nodulated and good growth occurred in the check solution without any added nitrogen, and this apparently caused a rapid intake of cations and thus a lowering of the pH values. Likewise, there was less physiological action in the sodium nitrate solution, and consequently less change in the hydrogen-ion concentration of the solution.

TABLE 3.-Reaction of nutrient solutions at end of changing period, expressed in pH units

Crop grown	pH of indicated nutrient solution					
	Check	Sodium nitrate	Ammonium sulphate ^a	Ammonium sulphate plus calcium carbonate	Urea 1	Urea 2
Grasses						
Timothy.....	6.6	7.8	3.8-6.5	7.1	7.3	7.4
Redtop.....	6.6	7.7	3.8-6.6	6.9	7.6	7.2
Orchard grass.....	6.6	7.5	4.0-6.4	7.5	7.3	7.3
English ryegrass.....	6.6	7.7	5.2-7.0	7.0	7.4	7.3
Kentucky bluegrass.....	6.5	7.5	4.0-7.0	7.0	7.0	7.0
Clovers:						
Red.....	5.6	7.0	5.5-7.0	6.9	5.8	6.0
Alsike.....	5.6	7.0	5.4-7.0	7.0	5.4	6.4
White.....	5.6	7.1	5.4-7.0	6.9	5.6	6.5

^a Near beginning and end of the experiment. The first value represents the end point in the first stages of the experiment, when plant growth was vigorous, the second that near the close of the experiment when the plant had practically ceased to function.

DISCUSSION

The data in table 1 clearly show the superiority of sodium nitrate over the other nitrogen carriers used, in the production of dry matter in the grasses. In the case of the clovers, the use of urea increased the dry-matter content most. This holds for both urea 1 and 2, but is especially marked in case of urea 2, in which the solution was changed most frequently and ammonification was at a minimum.

With both grasses and clovers, in all cases ammonium sulphate alone was decidedly the most inferior of all the nitrogen carriers used. The addition of calcium carbonate to the ammonium sulphate culture for the purpose of buffering the reaction, increased the yield over that obtained by the use of ammonium sulphate alone by about 36 percent in the case of the grasses, and about 125 percent in the case of the clovers. Nevertheless, the yield when ammonium sulphate plus calcium carbonate was used was poor in comparison with that obtained when the other carriers were used. Figure 1 also gives a good idea of the growth of the plants in the different media.

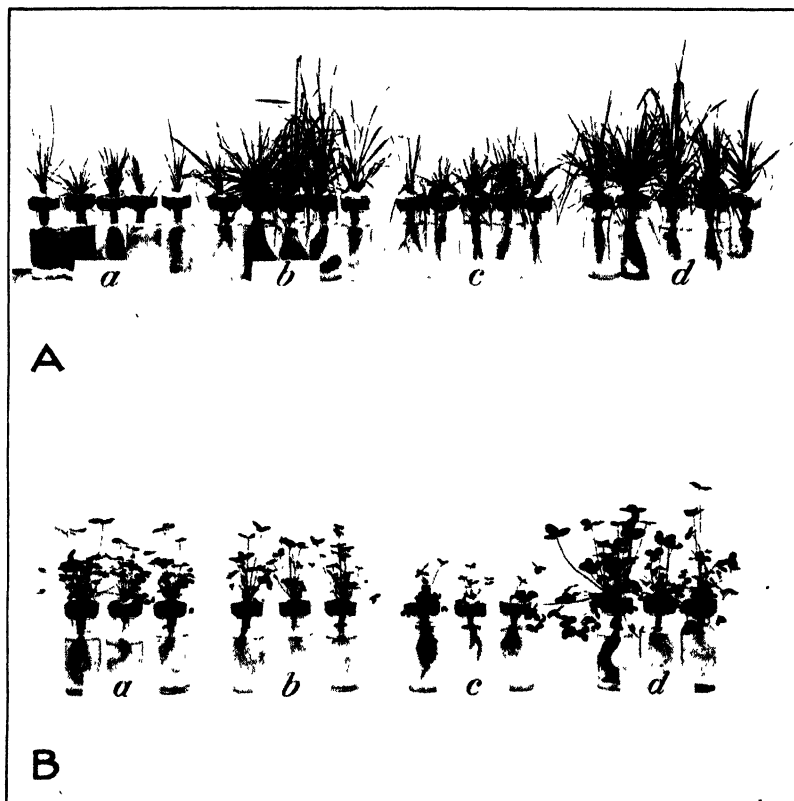


FIGURE 1—Grasses and clovers grown in unsterilized solutions and photographed when 32 days old. A, Left to right in each group. Kentucky bluegrass, English ryegrass, timothy, orchard grass, and red-top; B, left to right in each group. Red clover, white clover, and alsike clover. Nitrogen carriers, both series: a, Check; b, sodium nitrate; c, ammonium sulphate (without calcium carbonate); d, urea.

The differences recorded in table 1 and figure 1 were those existing at the close of the experiment. In the early stages of the experiment, for about 2 weeks, the growth of the grasses in the solutions containing ammonium sulphate was as good as, if not better than, in other solutions. On the clovers, however, the ammonium salt exerted an inhibiting effect almost from the start. About 2 weeks from the beginning of the experiment it became evident that the roots of both grasses and clovers in ammonium sulphate cultures were not normal in appearance. They became grayish brown and more or less slimy.

Eventually they became dark, and the roots of the clovers, which were more susceptible than the grasses, became almost black. Also, the roots had an odor of decaying organic matter. Practically the same root symptoms developed in the urea culture as in the ammonium sulphate culture, but were more marked in cultures containing urea 1 than in those containing urea 2 because of the greater amount of ammonia in 1. The maintenance of the reaction of the solution containing ammonium sulphate to practically a uniform level by means of calcium carbonate retarded slightly but did not eliminate the toxic action just described.

Table 2 shows that the percentage of total, water-soluble, and ammoniacal nitrogen in the tops and roots of grasses is considerably higher in plants grown in an ammonium sulphate medium than in other media used. The presence of calcium carbonate as a buffer made little difference in this relationship. In the case of the roots, urea caused about as much total and water-soluble nitrogen but not so much ammoniacal nitrogen to enter the plant. This would indicate that some of the urea was present in the roots in an unchanged form. On the grass tops the use of urea 1 produced effects more similar to those produced by the use of ammonium sulphate than did the use of urea 2. This can be explained by the fact that urea 1 solutions were changed less frequently than were those of urea 2, and consequently there was more ammonia present in urea 1; this was shown by chemical test. Nitrates were found only in plants grown in the nitrate solution, and in relatively small amount, which proves that nitrogen supplied in this form was better assimilated than were the other forms of nitrogen used.

According to their assimilability, the forms of nitrogen studied should be placed in the following decreasing order: For grasses: (1) Sodium nitrate (2) urea, and (3) ammonium sulphate; for clovers: (1) urea (2) sodium nitrate, and (3) ammonium sulphate. The order remained the same when the physiological acidity of ammonium sulphate was prevented by the presence of calcium carbonate, although growth was somewhat better under this condition. This leads to the conclusion that under the conditions of the experiment the toxicity of ammonium sulphate is due primarily to the accumulation of excess unassimilated nitrogen in the tissue, particularly the roots of the plant. This conclusion does not preclude the possibility of an injurious effect of the acid produced by physiological action, but it places it in a subordinate position. One of the authors (2) arrived at the same conclusion in a previous study of the toxicity of ammonium salts by tobacco.

Other workers in the field of absorption of nitrogen from nutrient solutions have reported decay of roots when grown in media containing ammonium salts. For instance, Loo (5) reported this for maize and wheat but attributed it to the physiological acidity of the solutions.

It should be emphasized that the results and conclusions just stated apply only to solution cultures. The writers do not mean to state or imply that the same or closely similar results will be obtained by the use of ammonium sulphate or urea for grasses and clovers grown in soil. Opportunity for rapid nitrification and other salutary actions in the soil appear to make these materials safe and desirable for practical purposes.

POSSIBLE EXPLANATION OF THE DECAY OF THE ROOTS

Series of both grasses and clovers were grown from seeds under sterile conditions according to a method (4) previously used for tobacco. The plants were grown 146 days without changing solution, so slow was the growth (fig. 2). Because of certain difficulties encountered it was not possible to obtain quantitative data. The qualitative results obtained checked rather closely those obtained with the unsterilized cultures, with the following exceptions:

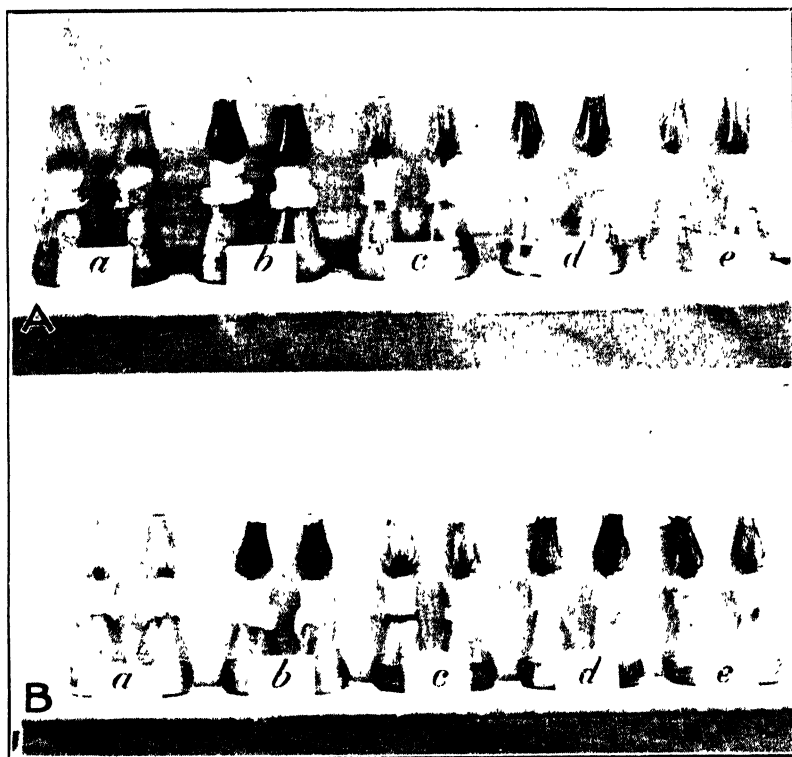


FIGURE 2.—Grasses grown in sterilized solutions and photographed when 146 days old. A, Timothy. B, redtop. Nitrogen carriers, both series. a, Check; b, sodium nitrate, c, ammonium sulphate, d, ammonium sulphate plus calcium carbonate, e, urea

(1) Physiological acidity developed in the unbuffered ammonium sulphate solution and was probably a more important factor than it was in the frequently changed but unsterilized cultures.

(2) Growth was almost, if not quite, as good in the ammonium sulphate solution buffered with calcium carbonate, as it was in the sodium nitrate solution.

(3) Roots of plants grown in cultures containing ammonium sulphate or urea did not turn grayish brown or black and decay, except where it was evident that infection entered from without.

The superior growth in the buffered ammonium sulphate solutions was probably due to the lack of root decay. This is considered a significant point. Under unsterilized conditions common decay

organisms have easy access to the roots, and by their action growth is retarded.

The question then arises as to why the roots decayed in unsterilized solutions containing ammonium salts and urea and did not decay to any serious degree in the check and nitrate solutions. The following hypothesis is advanced:

It has been shown that the nitrogen of ammonium sulphate and urea is not assimilated so completely or so rapidly as that of sodium nitrate. The excess nitrogen accumulates in the plant tissue, especially in the roots. A considerable portion of the excess nitrogen is water-soluble, some of it as ammonia, and probably a fair proportion is in amino or amide form. The carbon-nitrogen ratio is therefore probably much narrowed, especially in the fine roots. It is known from other investigations that organic matter breaks down particularly rapidly when the carbon-nitrogen ratio is narrowed down to about 15:1. It has been proved in the study of methods of making artificial manure from straw that ammonium salts and cyanamide are especially efficacious in promoting the decay of straw. Although these investigations have been with dead plant tissues, it does not seem unreasonable to think that the same method of disintegration of living root cells might occur, especially since some dead root tissue is always present.

SUMMARY

In a study of the assimilability of different chemical forms of fertilizer nitrogen, five grasses—timothy (*Phleum pratense*), reedtop (*Agrostis alba*), Kentucky bluegrass (*Poa pratensis*), orchard grass (*Dactylis glomerata*), and English ryegrass (*Lolium perenne*), and three clovers, red (*Trifolium pratense*), alsike (*T. hybridum*), and white (*T. repens*) were grown in nutrient solutions containing sodium nitrate, ammonium sulphate, and urea, respectively. In one group calcium carbonate was added to the solution containing ammonium sulphate for the purpose of buffering it against physiological change in reaction. In the early stages of growth the nitrogen of ammonium sulphate appeared to have been assimilated most rapidly and best by grasses, but in the experiment as a whole nitrate nitrogen was most readily and completely assimilated by the grasses, and urea by the clovers, as determined by the appearance of the plants, weight of dry matter produced, and chemical composition. In the later stages of growth, the plants grown in unsterilized ammonium sulphate solutions and in urea solution developed grayish-brown to black and more or less decayed roots. Plants grown in similar sterilized solutions did not develop this root trouble. It is suggested that the root trouble developed was due to the accumulation in the roots of unassimilated ammonia or other intermediate form of nitrogen, which narrowed the carbon-nitrogen ratio and made the root tissue susceptible to the attack of decay organisms. The order of assimilability of nitrogen is placed as follows: By the grasses: (1) sodium nitrate, (2) urea, and (3) ammonium sulphate; by the clovers: (1) urea, (2) sodium nitrate, and (3) ammonium sulphate. The addition of calcium carbonate to the ammonium sulphate solution did not change the order. The results obtained are not meant to be applied to field conditions under which nitrification and other salutary soil processes may considerably alter the condition of applied nitrogen.

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INJURY TO APPLE BY PETROLEUM-OIL SPRAYS¹

By PAUL A. YOUNG, *assistant botanist and bacteriologist*, and H. E. MORRIS, *botanist and bacteriologist*, Montana Agricultural Experiment Station²

INTRODUCTION

Petroleum-oil emulsions were used in attempts to control serious infestations of the fruit-tree leaf roller (*Cacoecia argyrospila* Walker) on apple in Bitterroot Valley of Montana during 1921 to 1925. Some of the emulsions injured the buds and leaves of apple (*Pyrus malus* L.), so it was desirable to secure more information about the effects of petroleum oils on plants, and to learn which oils could be used safely as emulsions on apple. Injury to plants probably is the limiting factor in the use of oil sprays.

This paper reports experiments carried on from 1926 to 1932, at Bozeman, Mont., and records mainly the studies on the character of the injuries to apple from oil sprays.

REVIEW OF LITERATURE

The literature on oil sprays is very extensive. Since Swingle and Snapp (34)³ reviewed many of the articles published before 1929, most attention is given in the present review to more recent papers.

Control of leaf-injuring insects is necessary to prevent decrease in the manufacture of food used by the apple trees. Proebsting (25) and Spuler, Overley, and Green (33) stated that decreasing the leaf area decreased growth in apple.

Oil emulsions commonly are classified as stable emulsions in contrast to unstable or quick-breaking emulsions in which the oil globules quickly accumulate as cream or free oil at the top of the water phase. In a miscible oil, the emulsifier is dissolved in the oil and makes the oil emulsify when stirred with water. Most of the soap emulsions and the emulsions made from miscible oils for commercial use have been stable emulsions. Casein has been used extensively in making unstable emulsions.

Writers disagree concerning the importance of stability as a quality of spray emulsions. Smith (30) favored the use of unstable emulsions. Swingle and Snapp (34) reported no practical difference between the covering powers of emulsions made from stock-oil emulsions and from miscible oils, while Anderson et al. (1) said that miscible-oil emulsions seemed to be slightly better in covering power.

Newcomer and Yothers (21) reported similar scalecidal effects from unstable caccinate-oil emulsions, stable miscible-oil emulsions, and stable boiled-soap-oil emulsions. Spuler, Overley, and Green (32)

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² The authors are indebted to the following oil companies for supplying the oils and information about the viscosities and sulphonatable residues of the oils: Standard Oil Co. of California, Standard Oil Co. of Indiana, Shell Oil Co. and L. Sonneborn Sons, Inc., of New York. Green (14)³ determined the sulphonatable residues of oils 5, 13, 15, 16, 21, and 28.

³ Reference is made by number (italic) to Literature Cited, 520.

observed that, despite the larger run-off, stable emulsions were as effective as unstable emulsions for insecticides in orchards. Robinson, Fisher, and Spuler (28) stated that high pressures used in spraying probably reduced the difference in the sizes of oil globules in stable and unstable emulsions. Spuler (31) and Spuler, Overley, and Green (32) warned against the building of too-thick deposits of oil by quick-breaking emulsions due to repeated sprayings or to the drifting of sprays by the wind. Green (14) reported the ability of one oil film to superimpose itself on another. Smith (29) illustrated the mechanism of deposition and accumulation of oil when sprays strike solid objects; this gave an explanation for differences in run-off from different emulsions. Bartholomew (2) reported the penetration of oil through both sides of citrus leaves.

Fear has been expressed that continual use of oil sprays might be detrimental to orchards, but evidence does not support this claim. Penny (24) reported no cumulative-oil injury in an orange orchard sprayed with oil during nine years. Quaintance, Newcomer, and Porter (27) also reported that cumulative-oil injury was not evident in thousands of orchards having eight or more annual sprays of oils on dormant trees.

Sulphonatable residues and viscosities of oils have been reported by many workers to be major factors in affecting oil injuries.

Oils for bud sprays should be 65 to 75 percent unsulphonatable, and have viscosities of 85 to 120 seconds. Robinson, Fisher, and Spuler (28) specified that oils for dormant use should have viscosities of 100 to 220 seconds, and unsulphonatable residues of 50 to 85 percent. Oils for summer sprays should have viscosities of 55 to 75 seconds and unsulphonatable residues of 85 percent or more. Newcomer and Yothers (22) made similar recommendations for summer oil sprays.

Some authors mentioned other factors affecting oil injuries. Ebeling (5) concluded that oil injuries to leaves were proportional to the percentage and heaviness of the oil, and inversely proportional to the amount of spreader and the sulphonation of the oil in the spray. Smith (29, 30) showed that uniform applications of quick-breaking emulsions required very thorough agitation.

Spuler, Overley, and Green (33) stated that the following factors influenced oil injuries to trees: Temperature, number of sprays, amounts of food and water, abundance of fruit, health, and varietal characteristics. Kelley (18) reported that the Grimes Golden apple tree transpired more rapidly and was affected more by oils than was the Jonathan apple tree which had a lower rate of transpiration. Felt (7) reported seasonal variation in susceptibility of plants to oil injury. Kelley (17) found that all of the oils that he used injured young leaves, while only the heavy oils injured old leaves.

Young (37) found that drought increased oil injury. Spuler, Overley, and Green (33) concluded that trees suffering from drought or malnutrition were especially injured by oils. Woodworth (36) stated that a dry condition of leaves facilitated penetration by oils. Browne (3) and Woodhams (35) advised that trees should have normal amounts of food and water before being sprayed with oils. Hartzell (15) warned that weak trees were especially susceptible to oil injury.

Knight, Chamberlin, and Samuels (19), and Spuler, Overley, and Green (33) reported that plants recovered more quickly from oils with low viscosities than from oils with high viscosities.

Browne (3) considered 32° C. to be near the maximum temperature for safe spraying with oils.

Ginsburg (10, 11, 12) observed that drops of some oils penetrated the lower sides of apple leaves within 1 or 2 hours, the rates of penetration being inversely proportional to the viscosities, and proportional to the amounts of the oils.

Ginsburg (11) tested many emulsions for injurious effects on apple, and mentioned the following symptoms of oil injury: Translucent, brown, and chlorotic leaf spots; early dropping of leaves and fruit; early blooming; altering of time of fruit ripening; russetting and decrease in size of fruit; and adhesion of dirt to oily surfaces.

Ginsburg (8, 9, 12) reported that oil-sprayed leaves were darker green, contained more chlorophyll, and dropped later than unoiled leaves, probably due to insect control. Penny (24) observed that heavy oils stimulated growth.

Woodworth (36) gave good suggestions in his classification of the effects of oils on plants. Young⁴ (37, 38) described symptoms of oil injuries in apple. Swingle and Snapp (34), and Farrar (6), described some symptoms caused by oils in orchards.

Spuler, Overley, and Green (32) illustrated apple flowers without leaves on twigs which had received a delayed dormant spray with a quick-breaking-oil emulsion, indicating that the oil killed only the leaves in the fruit buds on such twigs in this case.

Overholser and Overley (23) found that more than three oil sprays sometimes decreased size of fruit and set of buds. Newcomer and Yothers (22) illustrated symptoms caused by a heavy oil in three varieties of apple fruits.

Swingle and Snapp (34) summarized some reports on the effects of freezing following oil sprays; 5° C. was recommended as a minimum temperature for spraying. Newcomer and Yothers (21) concluded that freezing did not increase injury from oils applied in the spring and that delayed dormant oil sprays were injurious.

Spuler, Overley, and Green (32) correlated oil-spray injuries with bud development and recommended that oil sprays should not be applied during the critical period of bud development. Kelley (17) suggested that oils may partly break the dormancy of apple trees and thus make them more susceptible to injury by cold.

The uses of Oil Red O and Sudan IV to reveal oils in tissues were described by Proescher (26) and Haynes (16).

MATERIALS AND METHODS

Earlier work on petroleum oils as insecticides showed that the lubricating oils were more satisfactory than the kerosenes or the crude oils.

In the present investigations, 25 kinds of lubricating oils were tested in emulsions. The sulphonatable residues of these oils ranged from 0 to 41.2 percent; their viscosities ranged from 38 to 244 seconds as determined by the Saybolt universal viscosimeter (table 1).

⁴ YOUNG, P. A. SPRAY OIL PENETRATION ON APPLE LEAVES, LIMBS, AND FRUIT. 1929. (Abstract of papers, Amer. Soc. Plant Physiol., Des Moines, Iowa.) [Mimeographed.]

Twenty of the oils were tested as 1-percent oil emulsions. These and three other oils were tested in more concentrated emulsions to reveal symptoms of oil injuries and to determine the margin of safety for each oil.

Calcium caseinate, ammonia casein, and cresoap were the emulsifiers used. An electric stirrer was used in emulsifying the oils in tap water.⁵ The emulsions were used on the dates when they were prepared. Calcium-caseinate stock emulsions of one third oil were made by mixing 7.2 g of calcium caseinate in 640 cc of water and emulsifying 320 cc of oil in this mixture. Essentially, this method of making calcium-caseinate emulsions was devised by Burroughs (3a).

Ammonia-casein stock emulsions were made by mixing 1,500 cc of water and 22.32 g of casein, making this mixture alkaline with about 25 cc of NH_4OH (specific gravity, 0.9), and emulsifying 3,000 cc of oil in it. Spuler, Overley, and Green (32) mentioned ammonia-casein-oil emulsions.

Each cresoap-stock miscible oil was made by mixing 90 percent oil, 4.5 percent cresols, and 5.5 percent soap. Potassium oleate was used in preparing cresoap in 1926, and herring oil saponified with KOH was used in 1930 and 1931. For dilution each stock miscible oil was poured slowly on the sides of the vortex made in water by the electric stirrer. The emulsion was agitated for 1 minute after the miscible oil was added. Melander, Spuler, and Green (20a) described the method of making cresoap emulsions.

The oil emulsions were sprayed on an orchard of 86 Hibernial apple trees on the farm of the Montana Agricultural Experiment Station at Bozeman. This orchard was planted in 1921 but received little cultural care after 1924 mainly because irrigation water was unavailable. The east row of trees secured some water and food materials from adjacent fallowed ground, and these trees were normal. Many of the trees in the other five rows suffered from drought during each summer and were used in studies on the relation of drought to oil injury.

A bucket pump equipped with a Bordeaux nozzle, and using the maximum pressure, was used to drench apple leaves and twigs with emulsions. Each emulsion was sprayed on all the limbs of sprayed trees in 1926 and 1927; unsprayed trees were left as checks.

Important physiological differences occur in leaves on different sides and levels of apple trees, so, after 1927, single branches 1 to 3 m long were sprayed, and nearby limbs on the same trees were left as checks. Each emulsion was sprayed on 3 to 7 limbs on different trees on each date it was used. Spraying was done on calm days. Summer sprays were applied when temperatures were near 15° to 25° C., maximum temperatures usually near 30° occurring later during the same days. Relative humidities during these days usually ranged between 30 to 70 percent of saturation, with extremes of 14 to 87 percent. Winter sprays were applied when temperatures were about -5° to 10°.

⁵ Most of the emulsions used were prepared by J. R. Green, B. L. Herrington, and A. H. Johnson, of the Chemistry Department of the Montana Agricultural Experiment Station.

EXPERIMENTAL RESULTS

MAIN INJURIES CAUSED BY OILS IN APPLE TISSUES

Symptoms appeared in oil-injured leaves within different periods of time. Some symptoms became more prominent, while others remained unchanged or disappeared. Oils with large percentages of sulphonatable residues and oils applied in concentrations of 8, 16, and 100 percent were more injurious than less sulphonatable or concentrated oil emulsions.

Calcium-caseinate-oil emulsions caused angular mottling and translucence in leaves within a few seconds or hours after spraying as the first evidence of injury. These and other emulsions caused chlorotic spots within a few hours or days. In mild injuries translucence and chlorosis did not become more severe and even disappeared.

Severe injuries were characterized by wrinkling, general chlorosis, and hypophyllous⁶ browning, silvering, and purpling. Some oils caused epiphyllous purple spots. Cresoap emulsions caused epiphyllous white and tan spots in many leaves.

Translucent and chlorotic spots caused by abundant oils commonly developed into large necrotic brown or dry-green leaf spots, many of which had brown or purple borders. When such spots became very large or numerous, many of the affected leaves fell one to many weeks before the uninjured leaves. Brown spots severely decreased the functions of the leaves. Severe premature defoliation commonly was followed by the dying of many of the buds and some of the bark on attached twigs.

DETAILED DESCRIPTION OF SYMPTOMS CAUSED BY OIL SPRAYS

Oil sprays caused many different symptoms. Brief descriptions and illustrations of some oil injuries are recorded in literature, but as few symptoms have been described in detail, it seems desirable to record the symptoms observed.

The most common symptoms caused by oil emulsions, and the sulphonatable residues and viscosities of the oils that caused them, are given in table 1. It was decided that certain of the oils are too toxic for safe commercial use on apple leaves. The conclusions thus expressed are supported by unpublished data on the effects of undiluted oils on apple.

The comparative degree of injury caused by each oil was determined by the proportion of the injured and the uninjured parts of the sprayed leaves. The degree of oil injury and severity of each symptom is designated by one of the following numbers:

- 0 means no injury or symptom seen.
- 1 means traces of injuries and symptoms.
- 2 means moderate injury and common and prominent symptoms, sometimes economically serious.
- 3 means severe economic injury and abundant and severe symptoms.
- 4 means buds killed besides the symptoms included under 3; many of the attached twigs were killed.
- means symptom not considered.

⁶ The words "hypophyllous," "epiphyllous," and "amphigenous," have meanings similar to those of the corresponding Latin words as defined by Clements (4). Hypophyllous symptoms occur mainly in spongy parenchyma, and epiphyllous symptoms occur mainly in palisade parenchyma of apple leaves. Most symptoms and many parasites are in tissues instead of on them.

TABLE 1.—Symptoms of injuries most frequently appearing in apple leaves as a result of spraying with petroleum oils

Oil no	Name of oil	Portion sub-phonatable		Viscosity	Oil in spray		Duration of test	Yrs	No sprayed limbs	Usual degree of injury ^a	Premature defoliation ^a	Large, dark brown spots ^a	Epiphyllous purple spots ^a	Epiphyllous white spots ^a	Hypophyllous browning ^a	Chlorosis ^a	Rolling and wrinkling ^a	Oily, angular mottling ^{a, b}
		Pct	Secs		Pct	Yrs												
3	Standard-Oronite Tech. 13006R.....	5	100		1 1 2 4 8 16 1	1 1 2 3 3 19 2		17 340 27 45 33 19 28	0 0 0 1 2 3 0	0 0 0 2 3 3 0	1 0 0 2 3 3 0	0 0 0 2 2 2 0	0 0 0 2 2 2 0	0 0 0 2 2 2 0	0 0 0 2 2 2 0	0 0 0 2 2 2 0	0 0 0 2 2 2 0	0 0 0 2 2 2 0
4	Standard Mineral Seal 13007R.....	10	50		1 2 4 8 1	1 2 3 4 1		340 26 44 61 12	0 0 0 2 2	0 0 0 1 1	0 0 0 1 1	0 0 0 1 1	0 0 0 1 1	0 0 0 1 1	0 0 0 1 1	0 0 0 1 1	0 0 0 1 1	0 0 0 1 1
5	Standard 5 no 14510R..	41 6	110		4 8 1	1 1 1		13 13 4	3 3 3	3 3 3	3 3 3	1 0 0	2 2 2	2 2 2	2 2 2	2 2 2	2 2 2	2 2 2
6	Standard Diamond Paraffin.....	(^d)	99		1 2 3 1	1 1 1 1		60 60 60 60	0 0 0 2	0 0 0 2	0 0 0 2	0 0 0 2	0 0 0 2	0 0 0 2	0 0 0 2	0 0 0 2	0 0 0 2	0 0 0 2
7	Standard Atlantic Red	(^d)	244		2 3 4 1	1 1 1 1		60 60 30 30	1 1 2 3	0 0 2 2	0 0 2 2	0 0 2 2	0 0 2 2	0 0 2 2	0 0 2 2	0 0 2 2	0 0 2 2	0 0 2 2
11	Standard gas oil.....	40	38		4 8 1	1 1 2		30 30 35	3 3 1	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0
13	Shell 70 Pale, RI, 90.....	31 2	75		2 4 8 2	2 2 3 2		24 45 40 24	2 3 1 2	2 2 3 2	2 2 3 2	2 2 3 2	2 2 3 2	2 2 3 2	2 2 3 2	2 2 3 2	2 2 3 2	2 2 3 2
15	Shell 2.....	26 4	58		2 4 1	2 2 1		24 24 3	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0	2 2 0
16	Shell 3.....	10	55		2 4 8 1	2 2 1 1		26 27 3 2	1 2 2 2	1 2 2 2	1 2 2 2	1 2 2 2	1 2 2 2	1 2 2 2	1 2 2 2	1 2 2 2	1 2 2 2	1 2 2 2
18	Shell 5.....	9 8	108		2 1 1 2	2 2 1 1		26 27 13 28	1 2 0 0	1 2 0 0	1 2 0 0	1 2 0 0	1 2 0 0	1 2 0 0	1 2 0 0	1 2 0 0	1 2 0 0	1 2 0 0
20	Shell 105, H ₂ SO ₄ treated.....	2	90		4 8 1 2	3 2 1 1		10 12 27 4	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0
21	Shell 106, SO ₂ treated.....	15 6	53		2 4 8 1	2 2 1 1		27 38 41 20	0 3 4 1	0 3 4 1	0 3 4 1	0 3 4 1	0 3 4 1	0 3 4 1	0 3 4 1	0 3 4 1	0 3 4 1	0 3 4 1
22	Shell 107, H ₂ SO ₄ treated.....	2	51		2 4 1 1	2 2 1 1		25 41 24 14	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0	2 2 0 0
23	San Jose emulsion.....	10	50		2 4 8 1	3 2 1 1		17 27 13 36	0 1 2 0	0 1 2 0	0 1 2 0	0 1 2 0	0 1 2 0	0 1 2 0	0 1 2 0	0 1 2 0	0 1 2 0	0 1 2 0
24	Sonneborn Ammie 10080P.....	0	67		2 4 8 16 1	3 2 1 1 1		23 44 53 27 20	1 2 3 3 0	1 2 3 3 0	1 2 3 3 0	1 2 3 3 0	1 2 3 3 0	1 2 3 3 0	1 2 3 3 0	1 2 3 3 0	1 2 3 3 0	1 2 3 3 0
25	Standard 6 no 14819R.....	10	73		2 4 8 1	2 2 1 1		15 33 36 7	0 2 3 1	0 2 3 1	0 2 3 1	0 2 3 1	0 2 3 1	0 2 3 1	0 2 3 1	0 2 3 1	0 2 3 1	0 2 3 1
28	Standard 7, no 14776R.....	35 2	100		8 1 1	1 1 1		18 13 9	3 0 2	3 0 2	3 0 2	3 0 2	3 0 2	3 0 2	3 0 2	3 0 2	3 0 2	3 0 2
30	S. Ind Verdol, 67 percent oil.....	4	83		8 1 1	2 1 1		13 9 13	0 2 3	0 2 3	0 2 3	0 2 3	0 2 3	0 2 3	0 2 3	0 2 3	0 2 3	0 2 3
33	Shell E512, SO ₂ treated.....	12 8	52		8 1 1	1 1 1		5 11 4	0 3 0	0 3 0	0 3 0	0 3 0	0 3 0	0 3 0	0 3 0	0 3 0	0 3 0	0 3 0
34	Shell E513, H ₂ SO ₄ treated.....	13.3	51		8 1 1	1 1 1		10 5 0	2 0 0	2 0 0	2 0 0	2 0 0	2 0 0	2 0 0	2 0 0	2 0 0	2 0 0	2 0 0
35	Shell E514, SO ₂ treated.....	13.7	74		8 1 1	1 1 1		12 5 0	3 0 0	3 0 0	3 0 0	3 0 0	3 0 0	3 0 0	3 0 0	3 0 0	3 0 0	3 0 0
36	Shell E515, H ₂ SO ₄ treated.....	14	75		8 1 1	1 1 1		11 2 5	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0
37	Shell E516, SO ₂ +H ₂ SO ₄ treated.....	2.3	55		8 1 1	1 1 1		12 2 5	2 1 2	2 1 2	2 1 2	2 1 2	2 1 2	2 1 2	2 1 2	2 1 2	2 1 2	2 1 2

^a See text for explanation of numerical ratings.^b Pertains only to calcium-caseinate-oil emulsions^c These oils are not recommended for leaf sprays.^d High.

Under the name of oil, "Standard" refers to the Standard Oil Co. of California, except for oil 30, for which it refers to the Standard Oil Co. of Indiana.

SYMPTOMS AND SIGNS OF OIL INJURIES IN APPLE LEAVES

Premature defoliation means leaf dropping during one to many weeks before normal defoliation (table 1). Very toxic oil emulsions commonly caused serious premature defoliation, usually accompanying and following the development of dark brown spots. Brown leaf spots and premature defoliation were the most common symptoms of severe injuries by oils.

The outer one or two leaves developed by Hibernial apple buds normally were wrinkled and were only half as long as the normal, inner leaves. These dwarfed leaves were especially susceptible to oil injury in 1931, when they turned yellow and dropped from oil-sprayed limbs, while comparable dwarfed leaves on unsprayed limbs remained green and functional during subsequent weeks.

Two-percent oil emulsions of the most toxic oils and more concentrated emulsions of the other oils caused premature defoliation. New leaves developed within 71 days on 19 limbs after the old leaves had been killed by emulsions of 14 very toxic or concentrated oils. Oils did not kill the latent buds on such limbs.

Emulsions of 15 very toxic or concentrated oils killed apple leaves quickly. Abscission layers probably were incomplete in many of such leaves because they became brown and hung on the trees during 1 to 6 months. Many of the twigs died within a year.

Dark brown spots were a common symptom of severe injury and appeared within 5 to 60 days after leaves were sprayed with oils (table 1, and figure 1, *D*). The spots were amphigenous, oval to irregular, and 1 to 30 mm long. Emulsions of the most sulphonatable oils usually caused many brown spots within 5 to 10 days, while emulsions of the least sulphonatable oils more slowly caused few brown spots, and some of them caused no symptoms. Thus, an emulsion of 16-percent oil 24 (unsulphonatable) required 45 days to cause brown leaf spots. These spots became black 36 days later. In contrast, emulsions of 8-percent oil 13 (31.2 percent sulphonatable) caused brown and purple spots within 5 to 19 days.

Brown spots occupied 50 to 90 percent of the blades of badly injured leaves. Some spots were only hypophyllous when they first appeared, but usually later they became epiphyllous too. Oils enlarged many injured spots in leaves.

Dry green spots appeared instead of brown spots in the leaves of many limbs within 11 to 57 days after they were sprayed with 8-percent oil emulsions of nine kinds of oils. Oils in the affected spots probably delayed browning.

The dry green spots commonly had dark-brown or purple borders, irregular lines, or concentric-arc lines 0.5 to 2 mm wide (fig. 1, *D*). Similar borders and lines occurred on many dark brown leaf spots within 12 to 33 days after spraying with 4- and 8-percent oil emulsions in 1930. In some cases the distinctive brown or purple colors of the borders disappeared within 27 days, leaving only the plain brown spots.

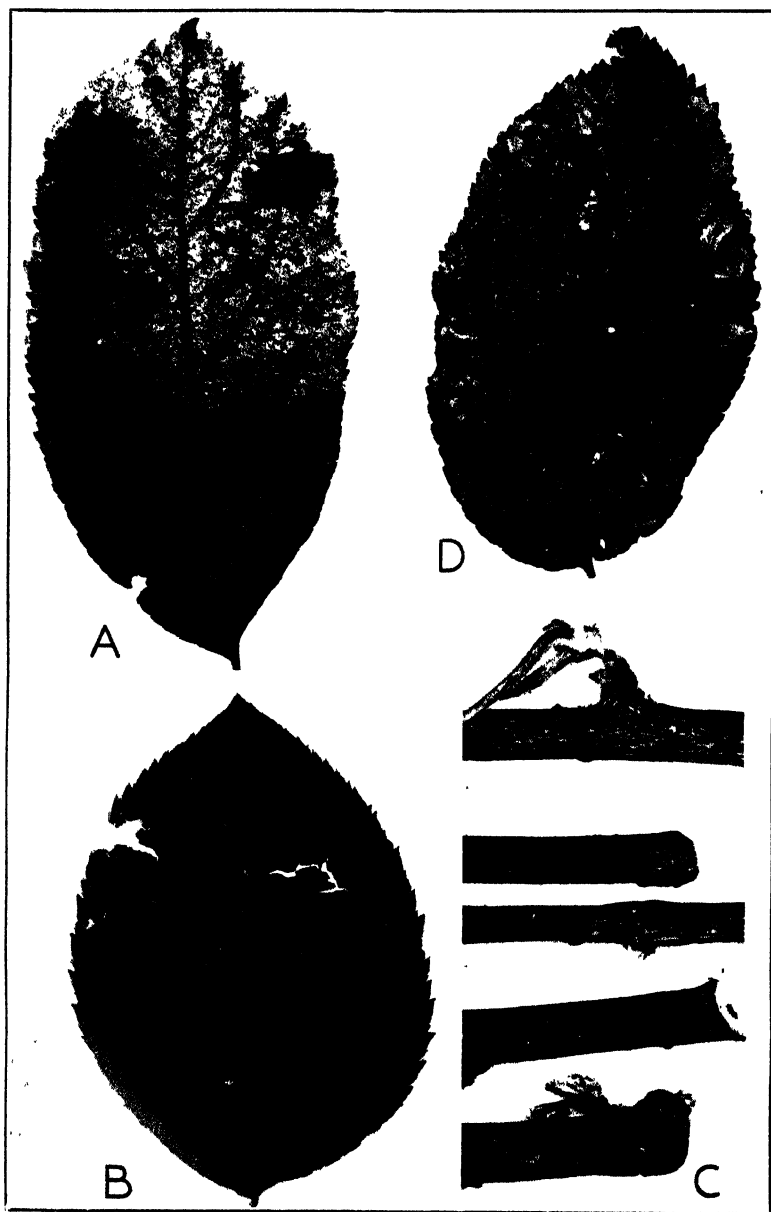


Figure 1—A, Angular mottling in apple leaf 38 days after it was sprayed with a calcium caseinate emulsion of 16-percent oil 24 on July 12, 1929. Gray parts of picture represent the oily, translucent parts of the leaf, while the black regions represent the normal, dark green, nonoily parts of the leaf. $\times 1$. B, White and tan epiphyllous necrotic spots in apple leaf 69 days after spraying with a cresoap emulsion of 8-percent oil 21. $\times 1$. C, Sharp, raised lines in apple twigs caused by 8- and 16-percent oil emulsions and pure oils applied to their leaves in 1930. The oils passed from the leaves into the twigs and killed the bark beyond the lines. Drying after excision caused the longitudinal wrinkles in the bark. $\times 1$. D, Typical brown borders on dry-green leaf spots in a leaf 60 days after spraying with a cresoap emulsion of 8-percent oil 3. $\times \frac{3}{8}$.

Cresoap emulsions of many oils caused white or light brown, angular or oval, epiphyllous spots 1 to 6 mm wide in apple leaves within 10 to 60 days after spraying (table 1 and fig. 1, *B*). Many of the spots had slightly raised, narrow, purple or dark brown borders. Although most of the spots were only epiphyllous, others were continuous with hypophyllous dark brown spots. Some of the epiphyllous spots had dark central dots (probably physiological). White and tan leaf spots were not seen on unsprayed limbs.

Cresoap emulsions of many oils in 4- and 8-percent concentrations caused white and tan leaf spots in 1930 and 1931, but only oil 13 (31.2 percent sulphonatable) caused them in a concentration of 1-percent oil. Such spots were not seen before 1930, although cresoap emulsions of many of these oils in concentrations of 2 and 4 percent were sprayed on apple leaves in 1929. Perhaps the greater drought injury in this orchard during the last 2 years affected the development of the leaf spots.

All the white and tan spots were caused by cresoap emulsions except some on one limb, which were caused by an ammonia-casein emulsion of 8-percent oil 4 (10 percent sulphonatable).

Delayed dormant sprays of 8- and 16-percent oil emulsions usually killed or severely injured the young leaves which they hit. Oiled parts of juvenile leaves turned light brown and died.

Angular, oval, or irregular epiphyllous, purple or reddish-purple spots 1 to 20 mm wide commonly resulted from sprays with oil 13 (31.2 percent sulphonatable) (table 1). Such spots usually were angular unless they were too small to affect all the epidermis bounded by small veins. The angular spots corresponded in size and shape with angularly mottled spots. Purple spots slowly became necrotic. Epiphyllous and hypophyllous purple spots usually did not occur in the same leaves.

Typical epiphyllous purple spots were caused only by oils 5, 7, 13, 15, 21, and 28, which are 15.6 to 41.6 percent sulphonatable. They were not caused by emulsions of less sulphonatable oils. This leads to the suggestion that the purple spots were caused by certain sulphonatable chemicals sufficiently concentrated only in the six oils listed. Emulsions of other oils with similar sulphonatable residues did not cause purple spots.

Epiphyllous purple spots served as a useful criterion for distinguishing some oils which were too toxic for commercial use on apple leaves. The purple spots were associated with dark brown leaf spots in late stages of development.

Angular, oval, or irregular, hypophyllous spots and sheens were common symptoms of mild injuries by oil emulsions, and these symptoms are included under hypophyllous browning in table 1. These spots and sheens were brown, purple, or silvery, and occurred separately and together in leaves. The epidermis on and between small veins was discolored. In some leaves only the veinal or interveinal epidermis was discolored. The discolorations often occurred in upward-rolled oil-injured leaves and were predominant within 2 cm of the edges. Usually, hypophyllous symptoms had no associated epiphyllous symptoms, but in some severe cases discolored hypophyllous spots were continuous with epiphyllous brown spots.

Chlorosis is a plesionecrotic symptom⁷ in which normally green tissues become abnormally light green or yellowish because of decreased amounts of chlorophyll. Instead of causing small chlorotic spots, some oils caused chlorosis of whole leaves or the parts of the leaves between the main veins. Circular mottled spots are included with interveinal and general chlorosis under Chlorosis in table 1.

Small mottled spots often occurred in leaves sprayed with cresoap emulsions of 4- to 16-percent oils. The spots usually were circular, chlorotic, and 1 to 5 mm in diameter. They probably were caused by emulsions entering the leaves, as they were not translucent like the spots caused by free oils.

Oils penetrated abundantly between the cells of leaves and quickly caused translucence of the invaded parts of the mesophyll (fig. 1, 4, and table 1). Nearly straight veins bounded the oily regions and made them angular. The amounts of oils entering the leaves determined

the sizes, number, and degrees of translucence of the angular mottled spots.

The oil sign, angular mottling or translucence frequently became a plesionecrotic symptom, angular chlorosis. Many angular mottled spots remained as chlorotic spots after they ceased to be evidently oily translucent.

Angular mottling was a characteristic effect of calcium-caseinate-oil emulsions. One- and two-percent oil-calcium-caseinate emulsions made fewer, smaller, and dimmer translucent spots than did more concentrated emulsions.

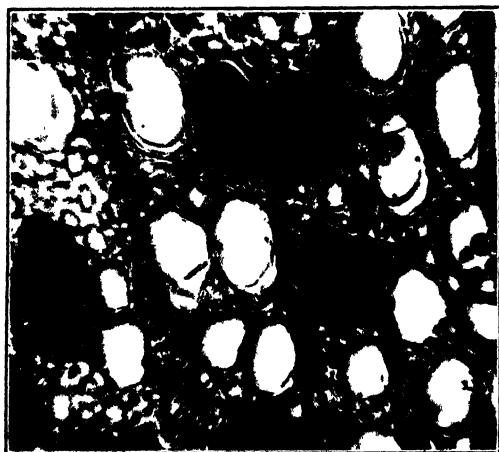


FIGURE 2.—Oil (photographed black) inside tracheae of an apple twig 109 days after its leaves were sprayed, with a calcium caseinate emulsion of 16-percent oil 24 × 328.

Translucent spots appeared soon after oil-calcium-caseinate emulsions dried on leaves.

One- to eight-percent oil-ammonia-casein emulsions occasionally caused angularly mottled spots. Probably they penetrated as free oils and also as emulsions so they caused less angular mottling than did the quick-breaking calcium-caseinate-oil emulsions which probably penetrated leaves mainly as free oils.

Cresoap emulsions of 1- to 16-percent oils rarely produced typical angular mottled spots. These stable emulsions presumably penetrated as emulsions. Kelley (18) and Ginsburg (11) reported penetration of leaves by emulsions.

Emulsions of 8- and 16-percent oils usually deposited on apple leaves and bark obvious films of oil which persisted 1 to 4 weeks after spraying. Such free oil, especially on the hypophyllous hairs of

⁷ Plesionecrotic symptoms are those characteristic of the stages of relatively slowly necrotic, abnormal protoplasmic degeneration preceding actual death of cells. Severe chlorosis, slow necrotic spotting, and purpling often are plesionecrotic symptoms.

leaves, held dust, making the affected parts dirty. Emulsions containing 1- to 4-percent oils usually did not make sprayed leaves evidently oily.

Sections of oil-sprayed leaves and their attached twigs revealed oil in and between the cells of many tissues (fig. 2). Abundant internal oils presumably were injurious before necrotic symptoms appeared.

Many oil emulsions caused abnormal degrees of wrinkling, upward rolling, and downward curling of sprayed apple leaves (table 1). Upward rolling of leaf margins often was associated with hypophyllous brown, purple, and silvery sheens.

Leaf dwarfing as a symptom of oil injury to buds is shown in table 2.

TABLE 2.—Symptoms of injuries most frequently appearing in apple buds as a result of spraying with petroleum oils

Oil no	Name of oil	Portion sul-pho-natable	Vis-cosity	Oil in spray	Limbs sprayed	Total degree of injury ^a	Buds killed ^a	Leaves dwarfed ^a	Re-tarded growth of leaves and flow-ers ^a
		Percent	Seconds	Percent	Number				
5	Standard 5, no 14510R . .	41.6	110	4	76	0	1	1	1
				6	26	1	1	2	2
				8	56	1	1	2	2
12	Shell Brown Neutral	27	115	16	59	3	2	3	2
				4	9	0	1	0	0
				6	9	0	0	0	0
13	Shell 70 Pale, R L 99 . . .	31.2	75	16	7	2	2	0	0
				4	7	0	0	0	0
				8	12	0	0	2	0
28	Standard 7, no 14776R	35.2	100	16	4	2	1	3	1
				4	35	0	1	1	1
				6	38	0	1	1	1
29	Standard (Ind), Dendrol .	30	83	16	39	3	2	2	2
				4	14	0	1	0	1
				6	4	0	1	0	0
				16	15	3	3	2	2

^a The numbers indicate the comparative amount of injury caused by each oil. 0=no symptoms seen, 1=a trace of injury, not commercially serious, 2=injury and symptoms common and serious, 3=symptoms abundant and destructive, causing severe injury.

^b Includes sprays on opening buds on May 14, 1929

Oil emulsions were sprayed on apple leaves 1 to 2 cm long in the orchard during blooming, on May 26, 1930. Four-percent oil emulsions of the oils noticeably dwarfed the leaves on some branches. One-percent oil emulsions did not definitely dwarf the leaves. Emulsions of 8- and 16-percent oils usually decreased or prevented subsequent growth of sprayed leaves. Leaf dwarfing was associated with necrosis of the leaves and flowers in many cases. These observations showed that 4- to 16-percent oil emulsions were commercially objectionable on juvenile leaves.

SYMPTOMS IN BUDS AND BARK

The opening and later development of buds was delayed by 16-percent oil emulsions sprayed on dormant buds (table 2). Opening buds were damaged by sprays of 8-percent oil emulsions. Latent buds developed on some limbs on which oils had killed the dominant buds. Emulsions of 4-percent oils rarely caused these symptoms.

Lethal quantities of oils entered some sprayed buds, causing them to become brown and dry. Lethal quantities of oils also passed from sprayed leaves into some of the attached twigs, causing the buds to die and the bark to become dry, brown, and shriveled. The rapidity and extent of the killing was generally directly proportional to the sulphonatable residue and concentration of each oil applied.

Some 8- and 16-percent oil emulsions killed the twigs attached to sprayed leaves. Such twigs typically developed sharp, narrow lines separating living and dead bark (fig. 1, *C*).

SYMPTOMS IN FLOWERS

Apple trees were sprayed on May 26, 1930, when in bloom. Most of the 4-, 8-, and 16-percent oil emulsions killed the sprayed flowers, turning the petals brown within a few days. Many oil-killed flowers remained attached to their twigs for a few weeks. Comparable 1-percent oil emulsions did not kill sprayed flowers.

SYMPTOMS IN FRUIT

Red-bordered black spots 1 to 3 cm wide occurred in the sunny sides of maturing apples on two limbs after spraying with emulsions of 8-percent oil 21 (15.6 percent sulphonatable), and 4-percent oil 4 (10 percent sulphonatable).

Russeted and cracked apples occurred on a limb sprayed with 4-percent oil 22 (2 percent sulphonatable). Abnormal fruit color was associated with oil sprays on many limbs in 1928. One- and two-percent oil sprays did not cause these symptoms.

OIL INJURIES TO APPLE BUDS

Oil emulsions were sprayed on apple buds and limbs in February, March, and April from 1928 to 1931, to determine which emulsions were safe for use on dormant buds.

No differences in effects of emulsions were found that could be attributed to differences in the emulsifiers.

Emulsions in concentrations of 4-percent oil rarely caused serious symptoms of injuries in sprayed buds, even after delayed-dormant sprays (table 2).

Emulsions of 6- and 8-percent oils frequently caused no symptoms in sprayed buds, especially in buds sprayed in February and March. However, many buds sprayed with 6- and 8-percent oils were seriously injured, especially following sprays on opening buds (table 2).

Emulsions of 16-percent oils usually were injurious. They were used only to reveal and make distinctly recognizable the symptoms of oil injuries.

QUALITIES OF EMULSIFIERS

Emulsifiers differ in their toxic effects and the ways in which they alter the miscibilities of emulsified-oil droplets with plant liquids. Emulsifiers probably facilitate the spread of oil in plants, and the mixing of oil in plant liquids. Emulsions may have the combined toxic effects of the oils and emulsifiers.

CRESOAP

Two percent of cresoap in water caused severe browning in apple leaves within 1 day and caused partial defoliation within a month. Concentrations of one fourth of 1 percent and of 1 percent of cresoap

in water caused no symptoms in sprayed leaves. A cresoap emulsion of 8-percent petroleum oil contains 0.89 percent of cresoap; this concentration of cresoap alone is probably not injurious. Concentrations of 1.78 percent of cresoap evidently were seriously toxic when they occurred in 16-percent oil emulsions of oil 3 (5 percent sulphonatable) and oil 24 (unsulphonatable) because these emulsions killed sprayed apple leaves within 1 to 30 days, while these oils in undiluted form and also in stock emulsions made with calcium caseinate and ammonia casein were much less injurious in comparable tests.

No deterioration of cresoap stock miscible oils was observed during 2 years, and dilution emulsions were made easily and accurately from them. Oil droplets 1μ to 5μ in diameter or smaller predominated in the emulsions. Four-percent cresoap-oil emulsions were stable for more than 16 months.

Stock emulsions of Solar Red Engine oil (Standard Oil Co.) and Diamond Paraffin oil were made in Illinois in 1923, according to the formula: 500 cc of oil, 125 cc of water, and 127.6 g of potassium-fish-oil soap emulsified by boiling and stirring with an electric stirrer. Dilutions of these stock emulsions resembled in stability and macroscopic appearance the cresoap emulsions made in 1927 to 1932.

CALCIUM-CASEINATE EMULSIFIERS

Calcium-caseinate-oil emulsions were unsatisfactory because they creamed and broke very rapidly, and apparatus was not available to provide sufficient agitation and pressure to secure uniform applications of oil. Sprayed leaves on three limbs were not injured by a suspension of 1 percent of calcium caseinate in water. A 1-percent oil-calcium-caseinate emulsion contains only 0.022 percent of calcium caseinate, so this emulsifier is not an important factor in the toxicity of calcium-caseinate-oil emulsions.

AMMONIA-CASEIN EMULSIFIERS

Sprayed leaves on three limbs for each concentration were not injured by the ammonia-casein emulsifier in water in the concentrations in which it occurs in 1-, 4-, 8-, and 16-percent oil-ammonia-casein emulsions. These concentrations of ammonia casein ranged from 0.016 to 0.256 percent, and were not considered to be important factors in the toxic effects of ammonia-casein-oil emulsions. Ammonia-casein emulsifiers made rapid creaming emulsions, but they were stable enough to permit uniform applications of oils.

SEMISOLID STOCK EMULSIONS

The semisolid stock emulsions of oils 23 and 30 were difficult to dilute accurately. Oil 23 was broken when sunlight warmed the containing can to about 50° C.

CONDITIONS AFFECTING OIL INJURIES

CORRELATED INJURIES CAUSED BY OILS AND DROUGHT

Nearly half of the oil-sprayed apple trees showed leaf symptoms of drought injury each summer. Observations in 1929 and 1930 revealed that the leaves on 102 limbs showed greater oil injury than corresponding oil-sprayed leaves on trees not showing symptoms of drought injury. No correlation was observed on 46 other limbs, be-

cause drought injury caused no apparent increase in oil injury. Thus, most of the evidence showed a direct correlation between injuries caused by drought and by 4-, 8-, and 16-percent oil emulsions of 12 kinds of oils sprayed on apple leaves. Observations in 1928 and 1931 also supported the conclusion that it is more dangerous to use oils on Hibernial apple leaves evidently suffering from drought than on normal leaves.

Water in and between cells theoretically constitutes the main barrier to the spread of petroleum oils in leaves, so oils are expected to spread less in normal leaves than in leaves suffering from drought.

TEMPERATURE, HUMIDITY, AND WIND

Daily records of temperature, humidity, precipitation, hours of sunshine, and velocity and direction of wind were kept at an official Weather Bureau station of the United States Department of Agriculture located nearly 400 meters southeast of the apple orchard. The records for the days when sprays were applied are not included because there was no correlation between the weather and the effects of the sprays.

Severe injuries were caused occasionally by 1- and 2-percent oil emulsions, perhaps affected by unusual conditions in the trees and weather. Oils more than 10 percent sulphonatable have small margins of safety even in 1- and 2-percent concentrations and may cause severe injuries. Unusual weather conditions occur frequently. Probability of occasional serious injury is a recognized hazard in commercial spraying.

Sprays froze on the trees on three dates, but the freezing made no apparent difference in the effects of the sprays. Unpublished data show that freezing usually did not break 4- and 8-percent oil-cresoap emulsions. The unstable calcium-caseinate emulsions broke and creamed so quickly that freezing probably was not important.

OTHER INJURIES

Wounds and dead spots in leaves and injuries made by mites, leaf hoppers, and aphids presumably facilitate penetration of oils, so enlarged wounds were a common symptom of oil injury.

In many cases, shaded leaves appeared to be injured less by oils than comparably sprayed leaves in sunlight.

The Hibernial apple orchard showed no evidence of cumulative injury by oil sprays during 6 years. The injuries caused by oil sprays were associated with individual oils applied on known dates.

DISCUSSION

Oil injuries in Hibernial apple leaves generally were correlated with the percentages of sulphonatable residues in the oils. Oils with high percentages of sulphonatable residues, in comparison with oils having low percentages of sulphonatable residues, usually caused a more rapid appearance of severe symptoms.

Exact correlations between sulphonatable residues and injuries were not expected, because sulphonatable chemicals presumably differ in their compositions and toxic effects. Thus, oils 16, 20, and 24 were more injurious to leaves than their sulphonatable residues of 10, 2, and 0 percent indicated.

The behaviors of such oils may be explained partly because some unsaturated compounds in oils are also unsulphonatable, so that even an oil showing no sulphonatable residue probably contained some unsaturated compounds. For example, some heavy olefines and parts of the naphthenic acids and sulphur compounds are not removed by sulphuric acid (Swingle and Snapp (34)), and some heavy naphthenes are not removed by sulphuric acid.

Eleven of the oils described in table 1 were tested by Green (14) who found that apple leaves and barley seedlings were similarly affected; they were most injured by the oils which were more than 5 percent sulphonatable. He determined the sulphonatable residues in these 11 oils and found close correlation between sulphonatable residues and injuries.

Green and Johnson (13) determined that three of these oils that were less than 16 percent sulphonatable decreased respiration of bean leaves, and three of them that were more than 16 percent sulphonatable increased respiration.

Oils have two main effects on leaves: (1) They plug stomata, intercellular spaces, and tracheae; and (2) the toxic parts of oils poison protoplasm. Apparently the toxic effects exceeded the physical effects of the oils more than 16 percent sulphonatable in the experiments cited in the preceding paragraph. Thus, oils with sufficiently concentrated sulphonatable chemicals had the predominating effect of increasing respiration in acting like other toxic chemicals as described by Kostychev and Lyon (20).

In these experiments there was no apparent correlation between degree of leaf injuries and differences in viscosities of spray oils varying between 50 and 108 seconds. However, Knight, Chamberlin, and Samuels (19) reported that leaves recovered more quickly from oils with low viscosities than from oils with high viscosities. Spuler, Overley, and Green (33) said that six applications of spray oils with viscosities of 70 to 120 seconds on heavily loaded trees reduced the sizes of apples. One application of oil injured Yellow Newtown apples.

Viscosities, volatilities, and distillation ranges are related qualities which are reported in literature as affecting the physiological effects of oils on organisms. Fairly straight-cut petroleum oils behave more uniformly in these qualities, so they are preferable to blended oils in sprays.

The viscosity of an oil is correlated with its rate of penetration and is determined at 37.7° C., a temperature which occurs in orchards, so viscosity probably is more significant than volatility or distillation range.

The relationship between volatilities determined at 50° to 100° C., distillation ranges determined at 288° to 399° (30), and volatilities of oils in orchards at 20° to 40° appears to be important and needs further investigation.

Nacent oxygen from photosynthesis in leaves may increase the oxidation of oils on and in leaves, forming oxides and peroxides. Thin films of oils exposed to air and sunlight may decompose and form acids injurious to leaves.

The chemical activity of an oil is increased, and its viscosity is decreased, by increasing its temperature. The speed with which an oil penetrates is increased by decreasing its viscosity. Hence, an oil at

25° C. is more active chemically and penetrates more rapidly than the same oil at 0°.

Agitation of sprayed leaves increases the amount of emulsion running off, so it decreases the amount of oil remaining and penetrating. The relation between the rates of evaporation and breaking of an emulsion determines the amounts of oil entering leaves in emulsified and unemulsified forms. Evaporation of water from emulsions on leaves is increased by increasing wind velocities and decreasing humidities.

Oil might delay or reduce the development of dormancy in predominant apple trees, and thus increase the hazard of winter injury. Hence, trees were not sprayed in October, November, and December.

SUMMARY AND CONCLUSIONS

The effects of petroleum-oil emulsions on apple were tested by spraying 25 kinds of oils on the leaves of 2,566 apple limbs during 7 years.

Oil sprays caused the following main symptoms in apple leaves: Premature defoliation; large brown spots; epiphyllous purple spots; hypophyllous brown, purple, and silvery spots; and chlorotic, translucent spots.

Epiphyllous purple spots served to distinguish some oils that were too toxic for commercial use on apple leaves.

The experiments showed that oils for applications to Hibernial apple leaves should not be more than 15 percent sulphonatable, and that oils only 5 to 10 percent sulphonatable were preferable. Oil injuries to apple leaves and buds usually increased with increasing amounts of sulphonatable residues in the oils. The different sulphonatable chemicals in the oils appeared to differ in their effects on apple. This probably explains why low concentrations of some oils were more injurious than higher concentrations of some other oils.

Oils usually were more injurious to drought-injured leaves than to normal leaves.

On the basis of injury to apple, the cresoap emulsifier was preferable to calcium caseinate, because cresoap emulsion of 1- to 8-percent oils were more easily diluted, more uniformly applied, and were less injurious to leaves.

The experiments showed that oils for application to Hibernial apple buds should not be more than 45 percent sulphonatable, and that oils only 30 to 40 percent sulphonatable were preferable.

Before using in orchards, each oil should be compared in concentrations of 16 and 100 percent with an unsulphonatable, similarly viscous oil on mature apple leaves to secure evidence of oil symptoms. Oils killing large parts of leaves within a week probably are too injurious for use on apple leaves. One- and two-percent oil emulsions which cause visible symptoms other than slight angular mottling in apple leaves are too injurious for commercial use on leaves. The speed with which an oil kills cells or causes plesionecrotic symptoms is an important index of its toxicity as a spray oil.

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SELF- AND CROSS-POLLINATION STUDIES OF APPLE VARIETIES, PARTICULARLY ROME BEAUTY AND GALLIA BEAUTY¹

By FREEMAN S. HOWLETT

Associate horticulturist, Ohio Agricultural Experiment Station

INTRODUCTION

During the last decade several apple varieties of the Rome Beauty type, which show considerable improvement in color of fruit, have been receiving favorable attention. Gallia Beauty, reported by Gourley and Ellenwood (7)² to be a seedling of Rome Beauty, is now being planted in a number of fruit-growing districts, of which Ohio is a typical example.

The question has thus arisen as to the degree of self-fruitfulness of Gallia Beauty and its interfruitfulness with other varieties of the Rome Beauty type. In 1927 the writer (9) presented data indicating that Gallia Beauty was not an effective pollinizer of Rome Beauty. At that time only a few flowers were used in the experiments. In 1930 further preliminary results were published (12) substantiating the previous conclusion. It was also concluded that Gallia Beauty, like Rome Beauty, possesses the characteristic of self-fruitfulness to a greater extent than do other varieties studied at the Ohio Agricultural Experiment Station. The amount of fruit produced from selfing, however, was not sufficiently large to give full crops of fruit. However, in 1930, Knowlton (13) published data which seemed to indicate that Gallia Beauty was an effective pollinizer of Rome Beauty. But very recently (14) he presented further data confirming the conclusions here reached.

The primary purpose of the experiments reported herein was (1) to obtain more extensive data regarding the self-fruitfulness and interfruitfulness of Rome Beauty and Gallia Beauty, (2) to determine the extent of possible seasonal variation in the self-fruitfulness of Gallia Beauty, Rome Beauty, Baldwin, and Jonathan, and (3) to determine the comparative value, as pollinizers, of several varieties whose planting in commercial orchards is relatively recent.

EXPERIMENTAL PROCEDURE

Selected branches of vigorous trees were inclosed in large cheesecloth bags, just as the terminal flowers of the clusters were about to open. The methods used were practically the same as those described in a previous publication (12). In the selfing experiments usually the entire cluster was hand-pollinated. In the cross-pollination work two large lateral flowers were usually pollinated, all other flowers being removed. Emasculation, when practiced (as shown in tables

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² Reference is made by number (italic) to Literature Cited, p. 536.

1, 2, and 4), consisted only of a removal of petals and stamens. When the flowers were not emasculated, pollination was carefully done by means of a camel's-hair brush.

PRESENTATION OF THE DATA

The data from the self-, cross-, and open-pollinated flowers are presented on the basis of the percentage of flowers that set fruit. The set after both the first and second (June) drop is given in tables 1, 2, and 4. In these tables is also given the percentage of set of the open-pollinated branches after the excess fruits had been removed. In Rome Beauty and Gallia Beauty this thinning, as indicated in table 5, consisted almost entirely of the reduction of the number of fruits per cluster to one. In Baldwin, Delicious, Jonathan, and Turley, the clusters were also thinned to one fruit per spur, and in addition the fruiting points were spaced so that the minimum distance between adjacent fruits was 8 inches. The open-pollinated branches had usually set abundantly. Except as otherwise indicated in the tables, the fruits left on the open-pollinated branches after thinning represented, in the writer's judgment, the maximum which the branches should hold to maturity. This number expressed in percentage of the original number of flowers on the branch is termed a "full crop" for the particular tree concerned and can be readily compared with the percentage of flowers setting fruit, on the hand-pollinated branches.

SELF-POLLINATION OF ROME BEAUTY

The data presented in table 1 show that an appreciable number of fruits were obtained from selfing Rome Beauty. In 1930 one of two selfed branches gave 0.8 percent. In 1931 the set ranged from 1 to 5.2 percent on 6 branches of 4 trees; in 1932 on a similar number of branches of 2 trees, the set ranged from 0 to 1.4 percent. Usually the higher percentages were obtained from partly deflorated clusters.

TABLE 1. -Results of self- and cross-pollination of Rome Beauty, 1930-32

TREE 430, 1930					
Pollen parent	Treatment of flowers in cluster	Number of flowers	Percentage of flowers setting fruit		
			After first drop	After second drop	After thinning
Rome Beauty.	(None emasculated	370	1.4	0.8	
	do	100	2.0	0	
	(2 not emasculated	60	3.3	0	
Gallia Beauty.	do	52	0	0	
	(None emasculated	173	1.7	.6	
Jonathan.	do	122	44.3	24.6	
Open pollinated.		695	49.3	32.7	9.6
TREE 324, 1931					
Rome Beauty.	2 not emasculated.	136	13.3	5.1	
Gallia Beauty.	do	118	.9	0	
Golden Delicious.	do	330	21.2	11.6	
Open pollinated.		1,127	49.2	13.2	9.5

TABLE 1.—Results of self- and cross-pollination of Rome Beauty, 1930-32—Contd.

TREE 325, 1931

Pollen parent	Treatment of flowers in cluster	Number of flowers	Percentage of flowers setting fruit—		
			After first drop	After second drop	After thinning
Rome Beauty	2 not emasculated	134	23.1	2.2	
Gallia Beauty	do	154	4.5	0	
Golden Delicious	do	156	0	0	
Open pollinated	do	178	2.8	2.8	
		1,001	51.8	10.5	8.3

TREE 326, 1931

Rome Beauty	2 not emasculated	211	7.6	5.2	
Gallia Beauty	do	119	1.7	9	
Melba	do	135	5.9	1.5	
Open pollinated	do	108	11.8	13	
		1,110	44.4	10.4	8.1

TREE 430, 1931

Rome Beauty	None emasculated	306	1.0	1.0	
	do	102	1	1	
	2 not emasculated	129	5.4	5.4	
	do	33	0	0	
	do	131	5.4	5.4	
Gallia Beauty	2 emasculated	81	1.2	0	
	do	99	4.2	4.2	
	do	156	.6	.6	
	do	105	0	0	
Rome Beauty, No. 73	do	114	2.6	2.6	
	do	186	0	0	
Jonathan	do	103	18.5	18.5	
Starking	do	128	6.3	6.3	
Open pollinated		1,172	62.8	20.2	12.5

TREE 326, 1932

Rome Beauty	None emasculated	140	1.4	1.4	
	2 emasculated	20	0	0	
	do	72	1.4	1.4	
	do	75	2.6	2.6	
Gallia Beauty	do	65	0	0	
	do	39	0	0	
Red Spy	do	59	23.7	23.7	
Golden Delicious	do	39	46.1	36	
Open pollinated		660	24.3	21.6	11.7

TREE 430, 1932

Rome Beauty	None emasculated	277	1.1	.7	
	do	415	.7	0	
	2 emasculated	102	11.7	8.8	
	2 not emasculated	112	2.7	2.7	
Gallia Beauty	2 emasculated	108	0	0	
	do	69	0	0	
Rome Beauty No. 73	do	108	0	0	
Golden Delicious	2 not emasculated	64	54.7	46.8	
	2 emasculated	65	7.7	7.7	
Open pollinated		1,035	30.9	28.9	16.8

In no case was the number of fruits produced equivalent to a full crop. In 1930 the set of fruit on the open-pollinated branches after thinning was 9.6 percent; in 1931 the set on the open-pollinated branches ranged from 8.1 to 12.5 percent and in 1932 from 11.7 to

16.9 percent. If the degree of self-fruitfulness of the variety is expressed as the relation between the respective percentages of the selfed and open-pollinated branches, in 1930 the selfed limb produced 8 percent of a full crop. In 1931 trees 324, 325, and 326 gave 53, 26, and 64 percent, respectively, of a full crop. In 1932 the two trees used in the experiment produced 12 and 52 percent of a full crop, respectively.

SELF-POLLINATION OF GALLIA BEAUTY

The data presented in table 2 show that small but significant percentages of set were also obtained from selfing *Gallia Beauty*. On the one tree used in 1930 the set on three branches was 2.7, 5.7, and 6.3 percent; in 1931 the set in three branches of a companion tree was 0, 1.2, and 4.9 percent. In this case the higher percentages were obtained from partially deflorated clusters. In 1932 the set on seven selfed limbs of one tree ranged from 0 to 3.4 percent, while on another tree no fruit was obtained from selfing.

TABLE 2.—Results of self- and cross-pollination of *Gallia Beauty*, 1930-32

TREE 16, 1930

Pollen parent	Treatment of flowers in cluster	Number of flowers	Percentage of flowers setting fruit --		
			After first drop	After second drop	After thinning
<i>Gallia Beauty</i>	None emasculated	192	10.9	5.7	
	do	186	5.4	2.7	
	do	127	8.7	6.3	
	2 not emasculated	97	3.1	3.1	
<i>Rome Beauty</i>	do	77	1.3	0	
	2 emasculated	99	8.1	6.1	
	None emasculated	148	3.4	3.4	
<i>McIntosh</i>	2 emasculated	58	39.7	22.4	
	do	84	39.3	28.6	
Open pollinated		355	67.6	23.9	14.9

TREE 4, 1931

<i>Gallia Beauty</i>	None emasculated	255	0	0	
	2 not emasculated	183	7.1	1.2	
	do	153	13.1	4.9	
	2 emasculated	160	10.6	3.1	
<i>Rome Beauty</i>	do	92	2.2	1.1	
	do	114	.9	.9	
	do	136	3.7	3.0	
	do	144	.7	.7	
<i>Stark's Dark Red Rome</i>	do	106	11.3	5.0	
<i>Rome Beauty No. 73</i>	do	60	8.3	1.7	
	do	173	4.6	1.7	
<i>Golden Delicious</i>	do	218	12.0	6.9	
<i>Jonathan</i>	do	103	20.4	16.5	
<i>Starking</i>	do	117	40.2	30.9	
Open pollinated, branch 1		459	73.8	16.7	8.7
Open pollinated, branch 2		354	69.5	16.7	9.3

TREE 16, 1932

<i>Gallia Beauty</i>	None emasculated	498	4.4	1.6	
	do	494	9.1	3.4	
	2 emasculated	87	0	0	
	do	234	1.0	.4	
	do	175	2.1	.6	
	do	187	0	0	
	do	96	0	0	

TABLE 2.— *Result of self- and cross-pollination of Gallia Beauty, 1930-32—Contd.*
 TREE 16, 1932—Continued

Pollen parent	Treatment of flowers in cluster	Number of flowers	Percentage of flowers setting fruit—		
			After first drop	After second drop	After thinning
Rome Beauty	2 emasculated	171	0	0	
	do	142	7	0	
	do	209	0	0	
	do	128	0	0	
	do	93	14 0	6.5	
	do	122	0	0	
Rome Beauty No. 73	do	213	1 0	0	
Golden Delicious	do	160	6	6	
McIntosh	do	122	52 3	24 8	
Red Spy	do	95	49 4	29 4	
Open pollinated	do	107	49 5	19 6	
	do	138	72.4	49 3	
		1, 157		17 5	9.3

TREE 4, 1932

Gallia Beauty	2 emasculated	171	0	0	
	do	43	0	0	
	do	63	0	0	
Rome Beauty	do	77	5 2	1 3	
	do	79	0	0	
Red Spy	do	37	91 9	89 2	

The fruits produced by selfing were in no case equivalent to a full crop. In 1930 the set on the three selfed limbs was equivalent to 18, 38, and 42 percent of a full crop. In 1931 the maximum percent from selfing was equivalent to 54 percent, and in 1932 to 36 percent, of a full crop.

CROSS-POLLINATION OF ROME BEAUTY

The principal pollination work with Rome Beauty was designed to test the effectiveness of Gallia Beauty as its pollinizer. A variety supposedly a seedling of Rome Beauty, and known as "Rome Beauty No. 73", was also used as a pollinizer. This variety is almost identical in color to Gallia Beauty but blooms a day or two later.

With but one exception (one branch on tree 326 in 1932), no higher set was obtained from cross-pollinating Rome Beauty with Gallia Beauty than from selfing Rome Beauty. It is obvious that on those branches where the flowers were not emasculated some of the fruits produced might have been from selfed flowers. Where the flowers were emasculated, Gallia Beauty gave, in several instances, percentages as high as were obtained from selfing Rome Beauty. Rome Beauty No. 73 also produced similar results. On the other hand, Golden Delicious, Jonathan, Melba, Starking, and Red Spy were very effective pollinizers.

In view of the difference in the set of fruit produced by Starking and Jonathan as pollinizers of Rome Beauty tree 430 in 1931, it might be assumed that these varieties differ in their effectiveness as pollinizers of Rome Beauty. The writer is of the opinion that other data than the percentage of flowers setting fruit must also be considered before such conclusions can be drawn. On this point, Crane and Lawrence (3) have suggested that the germination capacity of the seeds should

be taken as an index of the value of good pollinizers. The writer is of the opinion that both seed number per fruit (empty and plump seeds to be separated) and the germination capacity of the seeds are necessary before one should conclude that certain good varieties differ significantly in their effectiveness as pollinizers. The number of seeds in the fruits obtained from the self- and cross-pollination experiments is given in table 3. There was obviously no significant difference between the number of seed in the fruits produced by Jonathan and Starking as pollinizers of Rome Beauty. On the other hand the number of seeds in the selfed fruits of Rome Beauty, as well as in those produced by Gallia Beauty as the pollinizer, ranged from three to four per fruit.

TABLE 3.—*Number of well-filled and empty or flattened seeds of self- and cross-pollinated fruits of different apple varieties, 1930 and 1931*

ROME BEAUTY, TREE 324, 1931

Pollen parent	Total fruits	Seeds per fruit		
		Well-filled	Empty or flattened	Total
Rome Beauty ..	7	3 3	0 1	3 4
Golden Delicious	19	6 2	1 0	7 2

ROME BEAUTY, TREE 325, 1931

Rome Beauty.	3	3 0	1 0	4 0
Golden Delicious	5	5 8	6	6 4

ROME BEAUTY, TREE 326, 1931

Rome Beauty	4	1 0	0	4 0
Melba	14	5 3	7	6 0

ROME BEAUTY, TREE 430, 1931

Rome Beauty.	10	3 4	0 1	3 5
Gallia Beauty	11	2 6	4	3 0
Rome Beauty No 73	3	4 0	0	4 0
Jonathan.	17	6 0	1	6 1
Starking.	7	6 3	3	6 6

GALLIA BEAUTY, TREE 16, 1930

Gallia Beauty..	24	4 9	.8	5.7
Rome Beauty.	10	4 5	0	4.5
McIntosh.	28	5 3	1.0	6.3
Open pollinated	136	7 0	.2	7.2

GALLIA BEAUTY, TREE 4, 1931

Gallia Beauty.....	9	3.2	0 3	3.5
Rome Beauty.....	12	2 8	.8	3.6
Stark's Dark Red Rome..	5	3 6	.2	3.8
Rome Beauty No. 73.....	2	1 5	.5	2.0
Golden Delicious.....	15	5.9	.7	6.6
Jonathan.....	14	7.4	.5	7.9
Starking.....	36	6 9	.3	7.2

TABLE 3.—Number of well-filled and empty or flattened seeds of self- and cross-pollinated fruits of different apple varieties, 1930 and 1931—Continued

DELICIOUS, TREE 279, 1931

Pollen parent	Total fruits	Seeds per fruit --		
		Well-filled	Empty or flattened	Total
Cortland.	50	6 4	0 9	7.
Gallia Beauty.	82	7 0	.3	7.3
Golden Delicious.	24	6 4	1	6 5
Jonathan.	48	6 2	.2	6 4

JONATHAN, TREE 445, 1931

Jonathan.	29	2 7	0	2 7
Starking.	47	3 9	.2	4.1

TURLEY, TREE 477, 1931

Turley.	1	1 0	1 0	2 0
Delicious.	10	3 4	2 7	6 1
Jonathan.	2	5 0	1 5	6 5

CROSS-POLLINATION OF GALLIA BEAUTY

One phase of the experiments was largely designed to test the effectiveness of Rome Beauty as a pollinizer of Gallia Beauty. Rome Beauty No. 73 and Stark's Dark Red Rome were also used as pollinizers.

The data presented in table 2 show that Rome Beauty, with the exception of 1 limb (tree 16 in 1932) never produced more fruit as a pollinizer of Gallia Beauty than Gallia Beauty gave on selfing. Rome Beauty in several instances produced as high sets on Gallia Beauty as did Gallia Beauty when selfed. Stark's Dark Red Rome and Rome Beauty No. 73 gave similar results. On the other hand, McIntosh, Delicious, Jonathan, Starking, and Red Spy were very effective pollinizers.

The number of seeds (table 3) in the fruits produced by the good pollinizing varieties was not significantly different. The seed content of the fruits produced by selfing and by crossing with Rome Beauty, Stark's Dark Red Rome, and Rome Beauty No. 73 was lower than that of the fruits produced by crossing Gallia Beauty with McIntosh, Delicious, Jonathan, Starking, and Red Spy.

SELF- AND CROSS-POLLINATION OF OTHER APPLE VARIETIES

ARKANSAS

Auchter and Schrader (1) reported that Grimes was not effective in producing fruit when used as a pollinizer of the Arkansas variety. This is the only case yet reported in this country where a variety of apple with highly germinable pollen has failed to be an effective pollinizer of a variety to which it is not closely related.

The data presented in table 4 confirm the conclusions of Auchter and Schrader. Delicious and Jonathan were very effective pollinizers.

The flowers pollinated by Grimes Golden failed to enlarge even slightly and abscised during the early part of the first drop. These

flowers were fixed and examined cytologically. Although the examination has not yet been completed, none of the embryo sacs showed any evidence of fertilization. When fertilization takes place in 1 or 2 ovules of an apple flower, its receptacle enlarges slightly before the flower abscises.

TABLE 4.—Results of self- and cross-pollination of various apple varieties, 1931

ARKANSAS, TREE 262					
Pollen parent	Treatment of flowers in cluster	Number of flowers	Percentage of flowers setting fruit —		
			After first drop	After second drop	After thinning
Delicious	2 not emasculated	319	38.9	10.6	
Grimes	do	290	1.8	0	
Jonathan	do	154	0	0	
Open pollinated	do	250	39.1	10.9	
		2,105	6.3	5.8	5.8
BALDWIN, TREE 395					
Baldwin	None emasculated	510	7.7	3.1	
Open pollinated	do	14	3.5		
		1,071		10.7	6.5
DELICIOUS, TREE 279					
Delicious	None emasculated	279	0	0	
Starking	2 not emasculated	202	0	0	
Cortland	do	213	62.4	29.6	
Galla Beauty	do	203	78.3	39.4	
Golden Delicious	do	191	28.2	14.3	
Jonathan	do	191	16.2	13.6	
Turley	do	109	41.3	22.9	
Open pollinated	do	138	0	0	
		1,171		12.4	6
JONATHAN, TREE 445					
Jonathan	None emasculated	450	3.1	1.1	
Minkler	do	855		2.8	
Starking	2 emasculated	249	8	0	
Turley	do	754	33.1	16.9	
Open pollinated	do	200	19.5	14.0	
	do	200	0	0	
		557	39.5	4.5	4.5
		605	54.9	11.2	5.8
TURLEY, TREE 477					
Turley	None emasculated	263	1.1	8	
Delicious	2 not emasculated	216	8.8	8.8	
Jonathan	do	103	23.3	13.6	
Open pollinated		1,002	13.7	11.3	

* Not full crop.

BALDWIN

The primary purpose of the Baldwin selfing experiments during the period 1923 to 1931 was to determine possible seasonal variation in the degree of self-fruitfulness. This variety has occasionally been considered one in which such variation might be sufficiently great to give full crops in some years.

The data for the year 1931, presented in table 4, show that on two selfed branches 3.1 and 3.5 percent of the flowers set fruit, while on the open-pollinated branch 6.5 percent of the flowers did so. The degree of self-fruitfulness of this variety was thus approximately 50 percent of a full crop. On the basis of the proportion of flowering to fruiting points (table 5), 27 and 36 percent of a full crop was obtained from these branches. This difference in percentage is due to the fact that a few of the selfed clusters had two fruits. These would obviously be included in the percentage of flowers setting fruit, but not in the data based on the relationship of flowering to fruiting points.

TABLE 5.—*Relation between flowering and fruiting points of self- and open-pollinated branches of different apple varieties, 1930-32*

ROME BEAUTY, TREE 430, 1930

Pollen parent	Total number of flowering points	Percentage of flowering points with fruits	
		After second drop	After thinning
Rome Beauty	74	4.1	4.1
Open	139	48.2	48.2

ROME BEAUTY, TREE 430, 1931

Rome Beauty	61	4.9	4.9
Open	24	4.2	4.2
	252	72.3	72.3

ROME BEAUTY, TREE 326, 1932

Rome Beauty	25	8.0	8.0
Open	141	71.6	65.2

GALLIA BEAUTY, TREE 16, 1930

Gallia Beauty	50	20.0	20.0
	56	8.9	8.9
Open	40	15.0	15.0
	92	57.6	57.6

GALLIA BEAUTY, TREE 16, 1932

Gallia Beauty	89	6.7	6.7
	93	16.1	16.1
Open	206	53.9	52.4

BALDWIN, TREE 395, 1931

Baldwin	110	13.6	13.6
	50	10.9	10.0
Open	185	44.9	37.8

JONATHAN, TREE 445, 1931

Jonathan	84	4.7	4.7
	156	15.4	15.4
Open	110	51.8	31.8

DELICIOUS

The work with Delicious was designed to test the effectiveness of certain varieties as pollinizers of Delicious, the cheesecloth-bag method being used.

The selfed branch of Delicious (table 4) produced no fruits. In the cross-pollination tests Starking proved useless as a pollinizer. Overholser and Overlay (19) very recently reported a similar result and also showed the reciprocal cross to be unfruitful. Turley was of no value, since its pollen had a very low germinability, and the tubes produced were almost functionless. On the other hand, Cortland Gallia Beauty, Golden Delicious, and Jonathan were effective pollinizers.

The data for seed content (table 3) showed no significant differences which would indicate that the good pollinizing varieties are unequal in their value as pollinizers.

JONATHAN

The pollination experiment with Jonathan was designed (1) to test the effectiveness of Minkler, Turley, and Starking as pollinizers, and (2) to ascertain the degree of self-fruitfulness of the variety in the same year, as compared with Rome Beauty and Gallia Beauty, the cheesecloth-bag method being used.

The data (table 4) show that 1.1 and 2.8 percent were obtained from two selfed limbs. A full crop was equivalent to 5.8 percent. On this basis the self-fruitfulness was approximately 50 percent of a full crop. Data published in 1930 (12) showed that self-fruitfulness was 33 percent of a full crop on the tree used.

Minkler and Turley were of no value as pollinizers. Minkler, like Turley, had pollen of low germinability and produced short, thick tubes. Starking was very effective in producing fruit.

MCINTOSH

McIntosh, selfed, gave a small percentage of set, which was clearly only a fraction of a full crop.

TURLEY

The experiment with Turley showed it to be similar to Stayman in possessing a low degree of self-fruitfulness (table 4). Delicious and Jonathan were effective pollinizers.

DISCUSSION OF RESULTS

SELF-FRUITFULNESS OF ROME BEAUTY AND GALLIA BEAUTY

The experiments reported in the present paper conclude 8 years of work with Rome Beauty and 4 with Gallia Beauty. As reported in previous publications and as shown in considerable more detail in the present paper, both Rome Beauty and Gallia Beauty produced an appreciable number of fruits from selfed flowers, but in no case was the percentage of fruit equivalent to a full crop. On the basis of comparison with open-pollinated limbs, the degree of self-fruitfulness approximated 25 to 50 percent of a full crop. These varieties can be termed "partially self-compatible."

THE GALLIA BEAUTY-ROME BEAUTY RELATIONSHIP

The results presented herein conclude 6 years of work using Gallia Beauty as a pollinizer of Rome Beauty and 4 years using Rome Beauty as a pollinizer of Gallia Beauty. Rome Beauty and Gallia Beauty produced approximately the same percentage of fruits when used as pollinizers of each other as did either variety when selfed. Rome Beauty No. 73 and Stark's Dark Red Rome were also partially effective in producing fruit upon Gallia Beauty and Rome Beauty.

It might possibly be expected by some that if Gallia Beauty is a seedling of Rome Beauty, as has been reported by Gourley and Ellenwood (7), that the two varieties should be interfertile. Rome Beauty No. 73, also reported to be a seedling, was only partially effective as a pollinizer. Apparently seedlings of Rome Beauty, which differ from the parent variety practically only in color of fruit, are not completely compatible. The Gallia Beauty-Rome Beauty relationship and the reciprocal cross are apparently an example of incompatibility similar to that reported by Crane and Lawrence (3, 4) with closely related varieties of sweet cherry (*Prunus avium*). In this case the incompatibility is only partial.

THE ARKANSAS-GRIMES GOLDEN RELATIONSHIP

On the basis of present evidence, the failure of the Arkansas-Grimes golden cross does not appear to be an example of incompatibility of the same type as the Gallia Beauty-Rome Beauty relationship. In the first place, there is no genetical or systematic evidence of a relationship between the two varieties. Furthermore, Nebel (18) has reported that the Arkansas is triploid having 51 chromosomes, and that Grimes Golden is apparently diploid. The writer has observed from a cytological examination of the megasporogenesis that chromosomal aberrations are abundant in the Arkansas variety. Possibly, this is a factor in the failure of syngamy and seed development in this cross.

THE DEGREE OF SELF-FRUITFULNESS OF BALDWIN

The results obtained in the study reported in this confirm those already published (9, 10, 12) by the writer in regard to the degree of self-fruitfulness of the Baldwin. In no case during the 9 years' work (1922 to 1931, inclusive) with the enclosed-tree, glassine-bag, or cheese-cloth-bag method has the degree of self-fruitfulness been equivalent to a full crop.

The Baldwin has been reported by a number of investigators, of whom Nebel (18) is one, as triploid. Its pollen is of very low germinability, and a large proportion of the tubes produced are short and thick. There is no doubt but that chromosomal aberrations are abundant during megasporogenesis. The poor germination of the seeds and the weak growth of the seedlings are indirect evidence of that fact. In view of its triploid nature, the high degree of self-fruitfulness of the variety is surprising. In fact, its self-fruitfulness is higher than that of any triploid variety yet reported in this country. It is apparent that the tendency of the Baldwin to develop fruits, even though the seed number per fruit may be very low, is a factor of importance in determining the degree of self-fruitfulness of the variety.

SELF- AND CROSS-POLLINATION OF DELICIOUS

Until recently the results of cross-pollination experiments with Delicious as the female parent have been unsatisfactory. Ballard (2), Dorsey (5), Haber (8), and Murneek, Yocum, and McCubbin (17) obtained very low sets with a number of varieties now known to be good pollinizers. Whitehouse and Auchter (20) were the first investigators to report good percentages of set. Since that time Luce and Morris (15), Marshall, Johnston, and others (16), Einset (6), Overholser and Overley (19), and the writer have obtained very satisfactory sets. The writer agrees with Einset, who reviewed the data on this point, that the results in these crosses support the conclusion that emasculation is very detrimental to the set of Delicious. In no case has the writer been able to get a satisfactory percentage of emasculated flowers to set fruit. Fortunately, emasculation is unnecessary in cross-pollination tests in this variety.

The writer (11) showed that competition between the flowers of a cluster is another factor of importance in determining the percentage of flowers setting fruit. It was shown that as the number of pollinated flowers of a cluster increased, the percentage which could set fruit very rapidly decreased.

The evidence presented very recently by Overholser and Overley (19), as well as that given in this paper, indicates that the Delicious and its principal "bud sports" are not interfertile. In fact, the incompatibility is almost complete. This might be expected in view of the almost complete self-incompatibility of Delicious. This failure of Delicious and its closely related varieties is an example of incompatibility similar to those reported by Crane and Lawrence (3) for the sweet cherry.

SEASONAL VARIATION IN DEGREE OF SELF-FRUITFULNESS

When it is stated that apple varieties differ from year to year in their degree of self-fruitfulness, it is tacitly assumed that possibly certain varieties which are not sufficiently self-fruitful for a full crop one year may in another year produce a full crop. The suggestion has been offered that temperature changes, by affecting pollen-tube growth, are of great importance in producing this variation. The writer does not disagree with the importance of temperature in regulating pollen-tube growth. Unquestionably, the degree of self-fruitfulness of a variety differs from year to year and from orchard to orchard. The effect of partial defloration on increasing the percentage of fruits developing from selfed flowers indicates that the degree of self-fruitfulness may also vary from branch to branch and from flower to flower. However, during the 10 years of continuous experimental work at the Ohio station, the degree of self-fruitfulness has never varied to the extent that full crops have been produced on any variety used in the experiments. It might possibly be expected that full crops could be produced occasionally on Jonathan, Baldwin, Rome Beauty, and Gallia Beauty, but it has not been evident in these experiments or in any others.

The greater percentages of set obtained from flowers in a partially deflorated cluster might be expected in view of the more favorable nutritional conditions. For determining the degree of self-fruitfulness of a variety, a method which allows the flowers to develop under the most favorable nutritional conditions would, in general, seem the best

one. It must be admitted, however, that in pollinating only two flowers per cluster, commercial orchard conditions are merely approximated. Only when early frosts have eliminated several flowers of a cluster are the conditions in commercial orchards identical, as far as nutrition is concerned, with the experimental defloration procedure. Since, even under the most favorable nutritional conditions, a full crop of fruit is not produced from selfed flowers, the writer concludes that no variety in Ohio possesses a sufficiently high degree of self-fruitfulness to warrant its being recommended for planting in solid blocks.

CORRELATION BETWEEN POLLEN GERMINATION AND THE VALUE OF VARIETIES AS POLLINIZERS

Throughout the pollination work covering the 9-year period, there has been no case in which a sample of pollen which germinated very poorly gave a satisfactory set of fruit when taken to the orchard and applied by hand. This has been true whether the pollen has been from a variety such as Stayman Winesap, which normally has poor pollen, or from a variety which usually has good pollen. This indicates that the media used in the pollen germination tests are satisfactory, and this also makes it possible, in many instances, to predict the value of a variety as a pollinizer before one has cytologically examined microsporogenesis.

It has now become known that most of the varieties of apples which regularly have pollen of very low germinability are triploid. Nebel (18) has reported in this group Baldwin, Arkansas, Rhode Island Greening, Gravenstein, and Tompkins King. The writer has determined that Stayman Winesap is triploid and that Turley is apparently so. Minkler, which failed to be of value as a pollinizer in these experiments, is also likely triploid. As soon as the chromosome behavior of the various varieties during microsporegenesis has been examined, their effectiveness as pollinizers can be predicted. There are still several varieties of some commercial importance which have pollen of very low germinability and which have not yet been examined during the meiosis of the pollen mother cells.

SUMMARY

The experiments reported in this paper are largely concerned with the self-pollination and cross-pollination of Rome Beauty and Gallia Beauty. Of secondary importance are some data from several other varieties, including Arkansas, Baldwin, Delicious, Jonathan, and Turley.

Rome Beauty and Gallia Beauty produced an appreciable number of fruits when selfed, but under the most favorable nutritional conditions only 25 to 50 percent of a full crop was produced.

Gallia Beauty and Rome Beauty were only partly effective as pollinizers of each other. Approximately the same percentages were obtained as when each variety was selfed.

Rome Beauty No. 73, supposedly a seedling of Rome Beauty, was also only partially compatible on Rome Beauty and Gallia Beauty. Stark's Dark Red Rome also was only partially effective as a pollinizer of Gallia Beauty.

McIntosh, Jonathan, Golden Delicious, Starking, and Red Spy were effective pollinizers of Rome Beauty and Gallia Beauty. Melba was effective as a pollinizer of Rome Beauty. No evidence was obtained indicating a difference in the effectiveness of these varieties as pollinizers of Rome Beauty and Gallia Beauty.

The failure of Grimes Golden to produce fruit on the Arkansas was confirmed. This it is to be noted is a triploid $\times$ diploid cross.

Baldwin produced an appreciable set of fruit when selfed. Under favorable nutritional conditions self-fruitfulness was 25 to 50 percent of a full crop.

Delicious was self-unfruitful, and Starking failed to produce fruit when used as a pollinizer of this variety. The combination Delicious $\times$ Starking and its reciprocal, together with the Gallia Beauty-Rome Beauty relationship and its reciprocal, are apparently examples of an incompatibility similar to that found in other fruits, notably the sweet cherry.

Jonathan, Gallia Beauty, Cortland, and Golden Delicious were effective pollinizers of Delicious. No evidence was obtained indicating a difference in their value as pollinizers.

McIntosh had a low degree of self-fruitfulness.

Jonathan flowers showed a degree of self-fruitfulness equivalent to 25 to 50 percent of a full crop under the most favorable nutritional conditions. Because of their low pollen germinability, Minkler and Turley were ineffective pollinizers of Jonathan or any other variety on which they were used. Cytological examination of Turley during meiosis indicated that it is likely triploid.

Turley, a variety of the Stayman Winesap type, had a corresponding low degree of self-fruitfulness. Delicious and Jonathan were effective pollinizers.

The varieties which consistently showed the highest degrees of self-fruitfulness during the course of the writer's experimental work over a period of 9 years are Baldwin, Jonathan, Rome Beauty, and Gallia Beauty. This self-fruitfulness, however, even under the most favorable nutritional conditions, has never been equivalent to a full crop.

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LOSSES OF VITAMIN A IN DRYING FRESH RAW CARROTS AND SWEETPOTATOES AND CANNED SPINACH¹

By G. S. FRAPS, *chief, Division of Chemistry*, and RAY TREICHLER, *assistant chemist, Texas Agricultural Experiment Station*

INTRODUCTION

The method of estimating vitamin A requires that it be fed to rats over a period of 8 weeks. Storage for much longer periods is sometimes necessary until the animals are available for the purpose. Some material may deteriorate during the period of storage. In any investigation of the vitamin A activity of fresh materials, it is important to know how to store them without appreciable loss of this vitamin.

Some workers have dried the fresh materials and used the quantity of vitamin A in the dried materials to estimate the quantity in the fresh material, or the effect of conditions on it, but very little evidence is available as to the effect of drying on the quantity of vitamin A originally present. A number of investigators have studied the effect of different methods of drying on the vitamin A in the product obtained by the various processes, but these investigations throw no light on the amount of vitamin A lost during the drying. Some comparison of fresh material with the same food canned by different processes have been made, but the fresh material was usually purchased as needed and was not a portion of the food which was actually canned.

Jones, Murphy, and Nelson² compared the vitamin A content of raw frozen oysters with that of the dehydrated product and found that vitamin A was largely destroyed by dehydration. Dutcher, Honeywell, and Dahle³ compared fresh milk brought daily to the laboratory with the same milk pasteurized and evaporated and reported a loss of about 10 to 30 percent of vitamin A. Morgan and her co-workers⁴ compared the vitamin A content of fresh fruits frozen and kept at a temperature of -17°C . with the dried or dehydrated fruit prepared by different methods. Some of the fruits were sulphured before drying and some were not. Peaches lost about 14 percent of their original vitamin A, apricots 49 to 84 percent, prunes 9 to 76 percent, figs 50 to 80 percent, and apricots 50 to 70 percent.

The present paper reports an experiment to determine the loss of vitamin A in drying carrots, spinach, and sweetpotatoes, as compared with the vitamin A content of raw carrots, canned spinach, and raw sweetpotatoes, respectively.

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² JONES, D. B., MURPHY, J. C., and NELSON, E. M. BIOLOGICAL VALUES OF CERTAIN TYPES OF SEA FOODS. II—VITAMINS IN OYSTERS (*OSTREA VIRGINICA*). *Indus. and Engin. Chem.* 20: 205-210, illus. 1928.

³ DUTCHER, R. A., HONEYWELL, H. E., and DAHLE, C. D. VITAMIN STUDIES. XVI. VITAMIN A IN EVAPORATED MILKS MADE BY VACUUM AND AERATION METHODS. *Jour. Biol. Chem.* 75: 85-94, 1927.

⁴ MORGAN, A. F., and FIELD, A. VITAMINS IN DRIED FRUITS. II. THE EFFECT OF DRYING AND OF SULFUR DIOXIDE UPON THE VITAMIN A CONTENT OF FRUITS. *Jour. Biol. Chem.* 88: 9-25, 1930.

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MATERIALS USED

A case of canned spinach of apparently uniform quality was obtained. Some of the cans were opened, the juice was drained off, and the spinach was dried in a vacuum at 100° C. for 12 hours. During the process of the feeding experiment, a can of the spinach was opened each week, the juice was drained off, and the moist material was fed to rats. The dry material was determined, and the moist sample was kept in the refrigerator except while being fed.

Raw carrots were purchased on the market, and a portion was kept in the refrigerator. The supply was not sufficient, and another lot was bought toward the end of the experiment. Part of the carrots first purchased were ground and dried immediately in a vacuum for 12 hours at 73° C.

Puerto Rico sweetpotatoes were purchased from a farmer. A portion after being ground was dried for 7 hours at 100° C. The rest were stored at room temperature. It is possible that there was some loss of vitamin A during storage at room temperature.

All dried samples were stored in dark-glass containers, and only enough to last 2 or 3 weeks was removed and ground for feeding the experimental animals.

RESULTS

The units of vitamin A were estimated by the Sherman-Munsell unit method as used in this laboratory and previously described.⁵

Sixteen groups of rats, 6 in a group, except one group that had 8, were used in carrying out the experimental work. The average gains in weight, together with the amount of the particular vegetable allotted per day to each group, are found with other details in table 1. The material being tested was usually fed twice a week.

The raw yellow carrots contained approximately 43 units of vitamin A per gram of raw fresh material containing 11.4 percent dry matter. Reduced to a dry basis they had approximately 377 units per gram. The vacuum-dried sample of carrots contained approximately 77 units per gram. Approximately 80 percent of the vitamin A in the carrots was lost during the drying process (table 1).

The raw yellow sweetpotatoes with 28.7 percent of dry matter contained approximately 20 units of vitamin A per gram. This, when calculated on a dry basis, is about 70 units per gram. The vacuum-dried sample of the sweetpotatoes had 50 units of vitamin A per gram. The destruction of vitamin A appears to be approximately 29 percent. Other samples of yellow sweetpotatoes examined for their vitamin A content contained 50 units per gram on the original moist basis.

The canned spinach contained 140 units of vitamin A per gram of fresh pressed spinach solids, containing, on an average, 14.7 percent of dry matter. Calculated to a dry basis, the canned spinach had 952 units of vitamin A per gram. The vacuum-dried spinach contained 333 units of vitamin A per gram. Thus the spinach lost 65 percent of its vitamin A content during the drying process.

⁵ FRAPS, C. S. VARIATIONS IN VITAMIN A AND CHEMICAL COMPOSITION OF CORN. *Tex. Agr. Expt. Sta. Bul.* 422, 46 p. 1931

TABLE 1.—Effect of drying in vacuo on vitamin A in carrots, sweetpotatoes, and spinach

Laboratory no	Material tested and treatment	Rate fed per day	Rats at start of test	Rats at end of test	Average gain or loss in weight in 8 weeks	Feed per unit of vitamin A	Vitamin A per gram feed	Loss of vitamin A
		Gram	Number	Number	Grams	Gram	Units	Percent
36027	Carrots, yellow, dried in vacuo.	0.006	6	2	1	0.013	77	80
35974	Carrots, raw, yellow, original	.02	8	6	11	.023	a 43	
37082	Sweetpotatoes, yellow, dried in vacuo	.026	6	6	49			
		.01	6	1	5			
		.02	6	4	27	.02	50	29
		.03	6	6	44			
		.02	6	0	0			
36042	Sweetpotatoes, raw, yellow----	.04	6	3	8	.05	b 20	
		.06	6	5	45			
		.0026	6	2	20			
37110	Spinach, canned, dried in vacuo	.003	6	4	30	.003	333	65
		.055	6	4	61			
		.008	6	3	57			
37086	Spinach, canned (moist solids).	.01	6	4	39	.007	c 140	
		.012	6	5	60			

^a 377 units of vitamin A per gram of dry matter

^b 70 units of vitamin A per gram of dry matter

^c 952 units of vitamin A per gram of dry matter

Carrots lost approximately 80 percent of their vitamin A during drying in a vacuum oven and sweetpotatoes about 29 percent. Although the dried materials were high in vitamin A content, they did not represent the original material, since there were serious losses in drying. These results indicate that green vegetables may be very high in vitamin A. The vitamin content of dried material may not be taken to represent the vitamin activity of the original moist material, as there may be serious losses in drying.

The results do not show whether or not there are losses in vitamin A in storing the fresh material, but they indicate that the stored fresh food, or the canned food, may be much higher in vitamin A than the dried material.

SUMMARY

Raw yellow carrots contained approximately 43 units per gram of fresh material containing 11.4 percent of dry matter, or 377 units per gram of dry matter. Vacuum-dried carrots contained 77 units of vitamin A per gram. The carrots lost approximately 80 percent of their vitamin A when dried. Canned spinach contained 140 units of vitamin A in the pressed solids which contained 14.7 percent of dry matter, or 952 units per gram of dry matter. Vacuum-dried spinach contained about 333 units vitamin A per gram. The spinach lost 65 percent of its vitamin A content in drying. Yellow Puerto Rico sweetpotatoes contained 20 units vitamin A per gram, with 28.7 percent of dry matter, or 70 units per gram of dry matter. The vacuum-dried sweetpotatoes contained 50 units per gram. The sweetpotatoes lost 29 percent of their vitamin A in drying.

The vitamin A content of dried vegetables may not represent the vitamin A content of the original moist material, as there may be serious losses in drying. Fresh green vegetables may contain much higher amounts of vitamin A than might be expected from tests of the dried material.

THE PREVENTION OF ANEMIA IN SUCKLING PIGS, WITH OBSERVATIONS ON THE BLOOD PICTURE¹

By T. S. HAMILTON, *associate in animal nutrition*, G. E. HUNT, *formerly assistant in swine husbandry*, and W. E. CARROLL, *chief in swine husbandry*,
Animal Husbandry Department, Illinois Agricultural Experiment Station

INTRODUCTION

That nutritional anemia may occur in suckling pigs under farm as well as under experimental conditions is now well known. The pig, however, is not the only class of farm animals subject to this disease. The possibility of the occurrence of anemia in calves has been recognized at the Kansas station (3),² and Becker, Neal, and Shealy (1) have shown that this disease is quite common in cattle in certain sections of Florida. Hamilton and Card (22) and Elvehjem and Hart (12) have produced severe anemia in chicks by feeding them milk on screen floors. Hart, Elvehjem, Kenmerer, and Halpin (16) later showed that the feeding of milk and yellow corn to chicks, from hatching, readily produced anemia. However, anemia does not seem to be of any practical consideration in poultry production, and according to the work of Thomas and Wheeler (34) the same seems to be true of sheep.

In the case of the pig, however, it is now recognized that, under present-day methods of swine management, the disease is responsible for the loss of no inconsiderable number of animals before weaning time. Any environmental, farm, or experimental conditions that restrict the little pigs to a diet of milk alone for a period of 3 to 5 weeks after farrowing will usually produce the disease. While the etiology of the disease is still somewhat in doubt, investigations during the last 10 years have established the fact that the inclusion in the milk diet of the pig of very small amounts of soluble iron and copper salts, or of iron salts alone, will entirely prevent the onset of the disease.

WORK OF OTHER INVESTIGATORS

Although Doyle, Mathews, and Whiting (10) quote Braasch as reporting an extraordinarily high death rate of pigs due to anemia, in Schleswig-Holstein in 1890, and McGowan and Crichton (25) call attention to the probability that some of the swine diseases reported in the literature in the past may have been, in reality, anemia, practically all the investigations concerned with this disease in swine have been carried out during the last 10 years.

McGowan and Crichton (25) recognized the disease in England prior to 1923. The external symptoms as well as the gross lesions

¹ Received for publication May 26, 1933, issued October 1933

² Reference is made by number (italic) to Literature Cited, p. 561.

and microscopic findings at necropsy described by these investigators have been confirmed by Doyle, Mathews, and Whiting (10), as well as by others.

In 1924 McGowan and his associates (24, 26) produced anemia in suckling pigs experimentally by confining them indoors, without exercise, and with access only to the iron-poor diet of the sow. McGowan concluded that the disease resulted from an iron deficiency in the diet. He said that the disease could be readily prevented or cured by the inclusion of ferric oxide in the sow's feed. It was pointed out that the favorable effect upon the pigs of this procedure was probably due, not to an increase in the iron content of the sow's milk, but to the small amount of iron actually consumed by the pigs from the iron-rich feed and feces of the sow. The practicability of such a procedure for preventing anemia may be questioned, however, since Hart and his associates (6, 17, 18) failed to obtain beneficial results from the administration of iron oxide directly to the little pigs, and Elvehjem and Hart (12) found that ferric oxide gave negative results in the attempted cure of anemia in chicks.

In 1927 Doyle, Mathews, and Whiting (10) reported the results of 3 years' work on anemia in swine at the Purdue station. These investigators were unable to obtain the favorable effects of feeding iron to the sows that were obtained by McGowan. A possible explanation is that the pigs may not have consumed sufficient amounts of the sow's feed to include the necessary iron. In the Purdue experiments the iron was given in the form of ferrous lactate added to the mineral mixture to the extent of 1 percent, and the mineral mixture made up 2 percent of the ration. It may, therefore, be calculated that each little pig would have to consume daily well over one half pound of the sow's feed in order to get 25 mg of iron, a daily dose which has been successfully used by subsequent workers.

The Purdue workers found that anemia was about four times as prevalent, and the death rate nearly four times as high, among pigs kept indoors as among those kept outdoors on concrete. These workers concluded at this time that some factors in outdoor conditions were responsible for the prevention of the disease.

In the next year's work on anemia at Purdue different lots of sows and pigs were fed liver meal, wheat-germ meal, iron lactate, copper sulphate, manganese sulphate, and liver extracts in the rations. None of these feeds, minerals, or extracts were reported to have prevented anemia (5). In a further study (33, *Rpt.* 42) of possible environmental conditions pertaining to the development and prevention of anemia in pigs, groups of sows with their litters were (1) confined in a central hog house, (2) confined in a central hog house and exposed 30 to 35 hours per week to sunlight filtered through common glass, (3) the same except that the sunlight was filtered through Correx D glass, (4) exposed for 4 hours daily to rays from a carbon arc lamp, (5) confined outdoors in concrete-floored pens, and (6) kept in a pasture lot with a small individual hog house. The results of weekly hemoglobin determinations indicate no severe cases of anemia in any of these groups, although the pigs on pasture were the only ones which returned to and maintained a normal level of hemoglobin in the blood after the second week. The pigs irradiated with the carbon arc lamp and the pigs exposed to the sun's rays filtered through

different kinds of glass were little, if any, better off than the group confined indoors without irradiation of any kind. The pigs kept outdoors on concrete floors had a slightly higher hemoglobin content than had those retained indoors, but in this case their hemoglobin level was less than 50 percent normal at the end of 5 weeks. Mathews, Doyle, and Whiting (29) have further shown that exposure to the rays of a mercury-vapor-quartz lamp did not prevent anemia in pigs. The ineffectiveness of the direct rays of the sun or of ultraviolet-ray irradiation of the pigs to cure anemia has been further proved by the Wisconsin workers (17, 18). The results of 5 years' work on anemia in pigs at Purdue, where 120 litters numbering about 1,200 pigs were used, have been summarized recently by Craig (7).

The results obtained by the Purdue workers are quite similar to those obtained by Schofield (32) in Canada. According to this investigator, anemia frequently developed in suckling pigs during the first 2 weeks of life and, further, the feeding of iron salts, or iron and copper salts to the pregnant sows failed to prevent the disease in the pigs. Pigs raised outdoors, however, were resistant to the disease.

Hart and his associates at Wisconsin were probably the first to use copper salts as a supplement to iron salts in the cure of milk anemia in pigs. A report of the work done at that station appeared in 1929 (17, 18). A solution containing soluble iron and copper salts given to the pigs by mouth in amounts equivalent to 25 mg of iron and 5 mg of copper daily was highly effective in curing the disease. Commercial iron (ferrie) sulphate solution alone was also highly effective; however, these workers attribute the beneficial effects of crude iron salts to copper contamination, without which, they maintain, iron would be ineffective for hemoglobin regeneration. Hart and his associates point out the fact that anemia is common in pigs only between the ages of 3 and 6 weeks. If pigs fail to contract the disease before they start eating solid feed, they rarely become anemic. Neither do late-farrowed pigs contract the disease when they have the opportunity to root in the soil and eat a small amount of green vegetation long before they ordinarily begin eating of the sows' feed. The explanation of these facts lie in the iron (and copper) present in the soil and in most feeds.

The spontaneous outbreak of the disease in a few litters in the experimental herds in the spring of 1929 at Illinois started some work on methods for prevention of the disease. Previous reports (15, 20) from this station have confirmed the results obtained by Hart and his associates. Dosing the little pigs with a solution containing iron and copper salts was highly effective in preventing and in curing the disease. Feeding iron and copper salts to the sow before and after farrowing apparently did not increase the content of these metals in the milk. Litters farrowed in a central farrowing house but removed to cindered pens within a few days after farrowing did not develop the disease. A convenient method of preventing the disease was found to be painting the udder of the sows once or twice daily with an aqueous solution of iron and copper salts to which had been added a little corn sirup.

The same beneficial effect of allowing the little pigs access to soil, pasture, or cinders out-of-doors has also been observed at Purdue by allowing the pigs access to blue-grass sod placed in the pen inside a central farrowing house (33, *Rpt.* 43).

Doyle, in still more recent publications (8, 9), calls attention to the fact that soil alone, placed in the pens with pigs inside a central farrowing house, prevents the disease, although the hemoglobin content of the blood of these pigs is not so high as when blue-grass sod is used. Sows may be confined inside small houses, but if the little pigs gain access to an adjoining pasture, anemia will not develop. Green feed free from soil, when fed to the sow and pigs, failed to prevent anemia in the pigs, according to Doyle.

The simple and sure method, suggested by the Illinois workers (15, 20) of preventing anemia in suckling pigs reared indoors, by painting the sows' udders with a solution containing iron and copper salts, has been successfully adopted by the Wisconsin (6), Kansas,³ and Cornell (27, *Rpt.* 43) stations. It has been found at the Wisconsin station that brushing the sows' udders once daily is sufficient. Also, if the dosing method is used, a weekly dose of 150 mg of iron (ferric sulphate) and 25 mg of copper (copper sulphate) is effective and saves time and labor.

Willman and McCay (27, *Rpt.* 44) at Cornell, during a second year's study of practical methods of preventing anemia in pigs, found that drenching the pigs twice a week with a solution of ferrous sulphate (copperas) and then placing 0.1 percent of the salt in a palatable grain mixture, accessible to the little pigs after they were 14 days of age, entirely prevented the disease. They also reported that the blood hemoglobin was slightly higher when the pigs were drenched than was the case when the sow's udder was painted three times daily. Drenching with a copper-free iron salt was found just as efficient as was drenching with the copper-contaminated iron salts.

Studies of practical methods of prevention of anemia in pigs were continued at the Illinois station during 1930 and 1931. A summary of these results was presented by the senior writer to the twelfth annual Illinois veterinary conference, held at Champaign-Urbana, February 10, 11, and 12, 1931. In the following month, Hastings (19) expressed the views of a veterinarian on the subject of nutritional anemia in pigs. These ideas were so contrary to the findings at the Illinois station, as well as at other laboratories, that some comments on that article and also some of the results of the 1930 and 1931 studies at the Illinois station were later made by Carroll and Hamilton (4). The present paper describes in detail the later developments in the study of methods of preventing anemia in pigs, a brief summary of which has appeared in annual reports from this station (21, 23).

EXPERIMENTAL RESULTS

During the spring of 1930, 62 litters of pigs were used in anemia investigations. The dates of farrowing ranged from early February till late April. All the pigs used were purebred from the Duroc-Jersey, Hampshire, Chester White, and Poland-China breeds. The sows varied in age, some were gilts and some were older sows. All pregnant sows were fed and cared for in accordance with good farm practice. All sows had been fed in dry lots. About a week before farrowing the sows were placed in the central farrowing house and kept in individual pens. The sows' rations were not supplemented

³ AUBEL, C. E. Private communication.

with iron or copper salts. The litters were divided into five groups or lots,⁴ the division being arbitrarily made by placing the first litter to farrow in lot 1, the second in lot 2, and so on.

All pigs were weighed individually at birth, at 4, and usually at 8 weeks of age. Records were kept of the apparent condition of all pigs, of all deaths prior to 8 weeks, and in addition, of the performance of all pigs from this investigation that were placed on subsequent feeding trials. In 17 litters blood samples and weights of individual pigs were taken at birth and at least weekly thereafter until the end of the period of observation, usually 8 weeks.

Blood samples were taken from the ears of the pigs. A flashlight held beneath the ear was found greatly to facilitate puncturing a blood vessel of proper size. Hemoglobin concentration was determined by the acid hematin method of Newcomer (30), the Newcomer disk being standardized by the Van Slyke oxygen-capacity method (35). All hemoglobin values are expressed as grams of hemoglobin per 100 cc of blood. Cell-volume⁵ determinations were made by the use of hematocrit tubes of 1-mm bore. Heparin was used as an anticoagulant. The method used is similar to that described by Went and Drinker (37) in their micromethod for blood-volume determinations. As stated above the litters of pigs were divided among five different lots.

The little pigs in lot 1 received no mineral supplement. The sows and pigs were kept indoors throughout the period of observation, usually 8 weeks. Each litter was confined in a small pen 8 by 10 feet, with concrete floor. Over part of the concrete was a board floor, and straw was used for bedding. The little pigs in each litter had access to the mother's feed and feces, although excess feed was not allowed to remain in the troughs, and the floors were kept reasonably clean. On one side of each pen was woven wire fencing, and the troughs were of cast iron.

The pigs in lot 2 received no mineral supplement. Within a day or two after farrowing the sow and litter were removed to an outside house with board floor. The houses were well made and were supplied with plenty of straw for bedding. The houses were placed in a corner of a pen 8 by 16 feet, with concrete floor. The little pigs had access to the sow's feed and feces as in lot 1 but not to soil or vegetation. On warm days the sides of the houses facing the run were opened, and, whether they wanted it or not, the pigs were given a sun bath.

Lot 3 was confined indoors as described for lot 1. However, the udders of the sows in this group were brushed three times daily, from farrowing until the end of the period of observation, usually 8 weeks, with a solution containing iron and copper salts. The solution used contained approximately 25 mg of iron and 5 mg of copper per cubic centimeter and was made as follows: 19.64 g of hydrated copper sulphate and 89.51 g of anhydrous ferric sulphate were dissolved in 500 cc of water, and this mixture was then added to an equal volume of corn sirup.⁶

⁴ Some slight modification of this plan was made in the case of a few litters, on which blood studies were not made because of the lack of space within the central farrowing house.

⁵ By cell volume is meant the percentage of cells in whole blood.

⁶ In certain litters in this group the treatment was modified as indicated in the figures. The composition of these modified supplements was as follows: The iron-sirup solution was the same as the iron-copper-sirup solution without the copper sulphate. The iron solution without copper and sirup contained 17 ounces of hydrated ferric sulphate in one half gallon of water.

Lot 4 was confined indoors as described for lot 1, and for the first 4 weeks after farrowing no supplement was available. From the fourth to the eighth week after farrowing the little pigs only had access to a palatable feed mixture which contained 0.1 percent of added crude ferric sulphate.

Lot 5 was also housed inside. During the first 4 weeks after farrowing the udders of the sows of this group were painted with the iron and copper solution as described for lot 3. From the fourth to the eighth week the udders of the sows were not painted, but the little pigs had access to a palatable feed mixture containing 0.1 percent of added ferric sulphate.

The results obtained are illustrated in figures 1 to 5, showing the trend from birth to the end of the period of observation, of the blood-hemoglobin concentration and the weights of individual pigs in litters from each of the five groups.

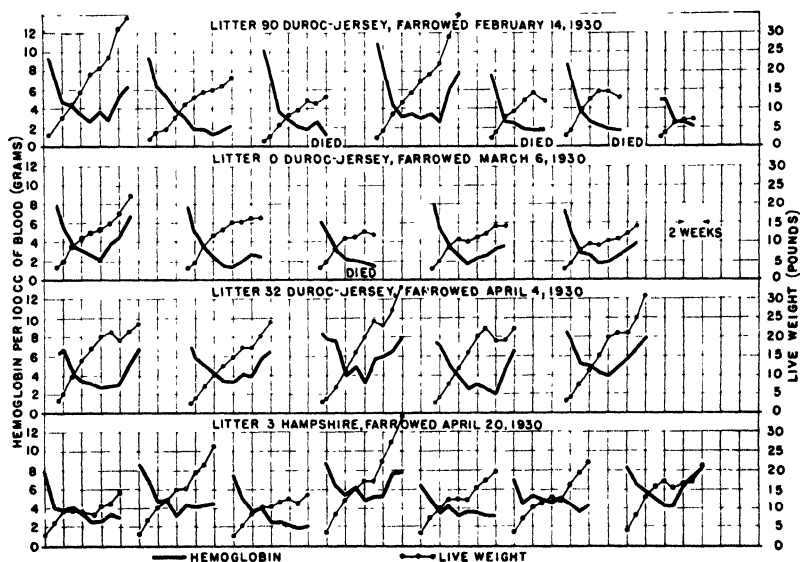


FIGURE 1—Blood-hemoglobin concentration and live weights of pigs in lot 1. The animals were kept inside and received no mineral supplement.

Figure 1 gives the curves for the hemoglobin concentration and live weights of pigs in four litters in lot 1. These pigs were confined indoors and received no mineral supplement. The chart is self-explanatory, and from it the following conclusions are drawn: (1) Pigs kept indoors and subsisting solely on their mother's milk are more likely to become anemic if farrowed during February and March than they are if farrowed in April; (2) anemia is likely to be more severe in the February and March litters than in April litters; (3) the mortality of anemic pigs is greater in the February and March pigs than in the April pigs; and (4) putting off the farrowing date to April does not insure pigs against anemia, if they are confined indoors and allowed access to a diet of milk alone.

Figure 2 gives similar data for three litters of pigs in lot 2. These pigs were kept under outdoor conditions and received no mineral

supplements. This figure indicates that early farrowed (February) pigs may become severely anemic and that the mortality may be very great, even though they are housed out of doors. Although the pigs in this group did not have access to soil, it is doubtful if this would have made any difference in the behavior of the February litter, since the weather was such as to cause the little pigs to eat and then huddle close together in the straw most of the time. For the March and April litters, however, there seem to be some beneficial effects of outdoor conditions, although there was access to neither soil nor vegetation. Sunlight as a factor seems to have been ruled out by the Purdue and Wisconsin workers. In view of what is now known concerning this disease, it seems reasonable to suppose that the environmental conditions during the late spring are such as to induce the little pigs to root around and eat small amounts of the sow's feed and feces at an earlier date than is the case with the early farrowed pigs.

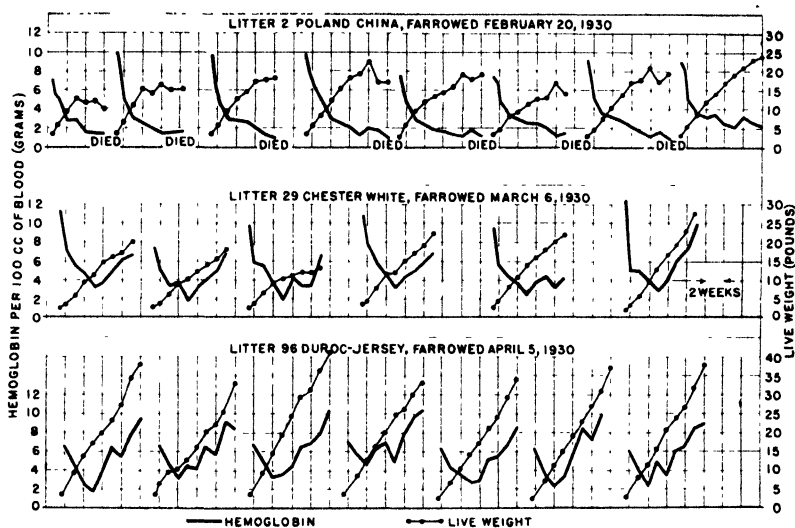


FIGURE 2 - Blood-hemoglobin concentration and live weights of pigs in lot 2. The animals were kept outside on concrete and received no mineral supplement.

The additional exercise induced also increases the little pigs' appetites perhaps beyond that satisfied by the milk supply, and this is another incentive to consume solid feed earlier. Along with the consumption of solid feed is the consumption of sufficient hemoglobin-forming essentials to enable the little pigs to escape the development of anemia.

Figure 3 gives the curves for the blood-hemoglobin concentrations and live weights for five litters in lot 3. This lot was kept indoors on concrete, but the milk diet of the little pigs was supplemented with iron, or iron and copper salts. The supplements were administered by painting the sows' udders with a solution containing these mineral salts. The first two litters, February and March litters, received the iron-copper-syrup supplement. Following the customary initial drop during the first few days, the hemoglobin concentration rose rapidly so that at the end of 2 weeks it had reached a nearly normal level.

Because of the possible toxic effects of copper sulphate, both to the little pigs and to the sow, and because it is questionable whether or not the little pigs as usually cared for need additional copper in their diets, data for two litters are presented to show the effects of the udder treatment when the copper is omitted from the solution. For the fifth litter, the concentration of iron was doubled and both the copper salt and the sirup omitted.

The data presented in figure 3 demonstrate the efficacy of iron and copper, or of iron salts alone, in preventing anemia in suckling pigs reared indoors. The udder treatment is shown to be a practical method of administering these mineral salts. That neither the sirup nor the copper salt is essential in the treatment is also indicated.

Figure 4 gives the blood-hemoglobin concentration and live weights of three litters of pigs in lot 4. In this group neither the sow nor the

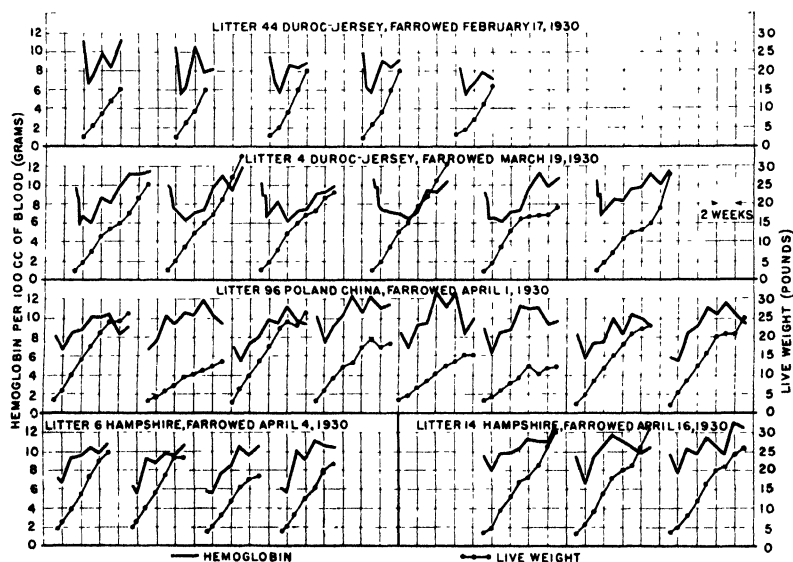


FIGURE 3.—Blood-hemoglobin concentration and live weights of pigs in lot 3. The animals received iron and copper, or iron supplements. Litters 44 and 4 Duroc-Jersey received the iron-copper-sirup supplement throughout the period of observation; litters 96 Poland China and 6 Hampshire received an iron-sirup supplement, without copper. Litter 14 Hampshire received an iron supplement only, without copper or sirup.

little pigs received any special treatment, other than their restricted confinement indoors on concrete, until the pigs were 4 weeks old, after which time the little pigs had access to a palatable feed mixture which contained 0.1 percent of added ferric sulphate.

Because of the unreliability of the treatment to which the pigs in this group were subjected, a more detailed description of the results obtained will be given. One litter was farrowed February 7. Twelve pigs were farrowed, but 1 died when it was 4 days old; the 11 pigs remaining all survived. At 17 days of age 9 of the 11 pigs were distinctly anemic, but at 59 days, when the test on this litter was discontinued, the average hemoglobin was 7.9 g. In contrast to the behavior of this litter is that of a litter farrowed 12 days later. This was also a large litter; 11 pigs were farrowed, but 1 died on the fourth day.

The average blood-hemoglobin concentration and live weight at birth were nearly identical for the two litters. While there were no deaths in the February 7 litter after the fourth day, there were five deaths, or 45 percent of the February 19 litter.

The third litter shown in figure 4 consisted of eight pigs farrowed March 20. Although the pigs in this litter had access to the iron-containing feed after they reached 4 weeks of age, they fared no better than the March litter in lot 1 which did not have access to the iron-rich feed. It may be noticed from the figure that the pigs in this litter all became severely anemic and that 5 of the 8 pigs died.

Figure 4 illustrates the fact that allowing the pigs access to an iron-containing supplement, which they may or may not consume, is of doubtful value. If the pigs have learned to eat solid food early enough (when 3 to 4 weeks old) the accessibility to an iron-rich supple-

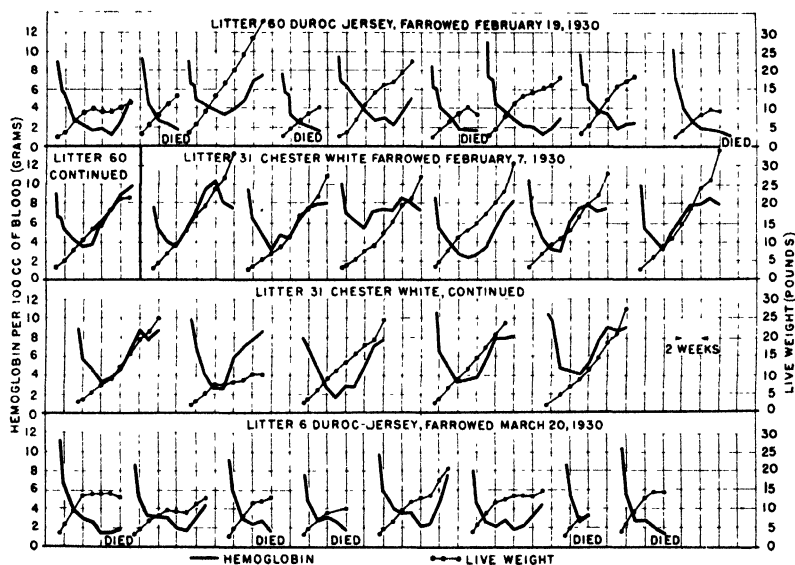


FIGURE 4 Blood-hemoglobin concentration and live weights of pigs in lot 4. The animals received no supplement the first 4 weeks, and then they had access only to feed to which 0.1 percent ferrie sulphate had been added.

ment will serve to counteract the onset of anemia. Even without the iron-rich supplement such pigs eat the sow's feces and feed and obtain sufficient hemoglobin-forming essentials to prevent severe anemia. It was noticed, particularly in the case of the February 7 litter, that the little pigs began nibbling the sow's feed before they were 3 weeks old. It may have been that the sow, being unable to furnish sufficient milk to satisfy the appetites of such a large litter, was responsible for this.

In contrast to the behavior of the pigs which began eating solid feed at an early age is the behavior of the pigs in litters farrowed February 19 and March 20. But few of the pigs in these litters were noticed to be interested in solid feed by the time they were 4 weeks old. Most of these pigs were then so anemic (the average hemoglobin was 2.23 and 1.56 g, respectively, for these litters)

that, although an iron-rich feed was available, they were too weak and indifferent to consume it. Forty-five percent of the February 19 litter and 63 percent of the March 20 litter died, while none of the February 7 litter died after the eighth day. The average weight of the three pigs left in the March 20 litter at 53 days was only 15.5 pounds.

The treatment for the pigs in lot 5 consisted in painting the sows' udders with the iron and copper solution, from farrowing until the pigs were 4 weeks old, after which time the pigs had access to the feed containing 0.1 percent added ferric sulphate. The litters were kept indoors throughout the test. The usual data for two litters in this group are given in figure 5. The excellent condition of the pigs, as judged by the hemoglobin concentrations and live weights, is obvious.

In addition to the blood studies made on the spring pigs just described, 15 summer and fall litters were studied also. The pigs were confined either inside (lot 1) or outside (lot 2) on concrete and received no mineral supplement. In general the blood was examined only

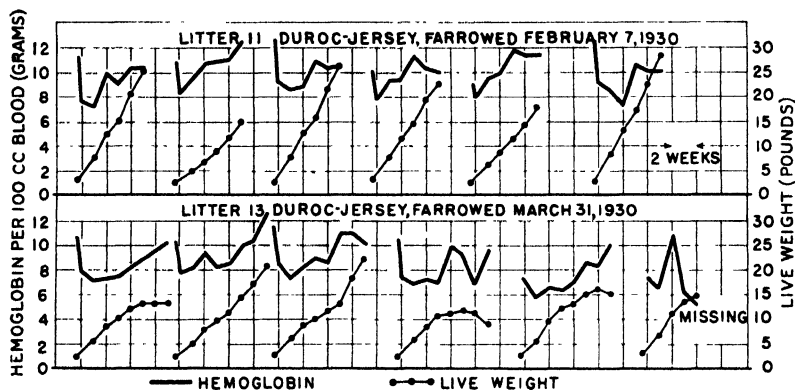


FIGURE 5.—Blood-hemoglobin concentration and live weights of pigs in lot 5. The animals received the iron-copper-sirup udder treatment during the first 4 weeks after farrowing; afterwards they had access only to a feed mixture containing 0.1 percent of added ferric sulphate.

at the end of the third and fifth weeks in order to determine the incidence and severity of the anemia developed under these conditions.

One litter of six pigs was farrowed July 6 and placed in lot 1 (inside, untreated). This litter failed to develop severe anemia. The average hemoglobin value at 22 days was 5.82 g and immediately thereafter showed an increase, so that at 36 days the average was 7.7 g.

Thirteen September litters were placed in the untreated lot 2 outside group. There were 71 pigs in these litters, and 14 (or 20 percent) had a hemoglobin concentration of 3.5 g or less at 3 weeks of age. Three litters containing 12 pigs were examined at 5 weeks of age, and the average hemoglobin concentration was 6.98 g. Only 1 of the 12 pigs was anemic at this time.

While several September litters showed an incidence of anemia of but 20 percent, late spring pigs or fall pigs may become severely anemic. This is illustrated by a litter of six pigs farrowed October 6, 1930, and confined on concrete under lot 2 (outside) conditions. The average hemoglobin concentration of the blood of this litter was, at 25 days, but 2.01 g. All six pigs were severely anemic, the maximum

hemoglobin concentration being 2.63 g. These pigs were immediately treated, and the test was discontinued.

In the spring of 1931, 3 litters, 1 farrowed March 25, 1 March 30, and 1 April 2, were used to study the effect of enforced exercise upon the incidence of anemia as determined by the blood hemoglobin. The procedure followed was to number each pig as soon after birth as possible and to divide each litter into approximately two equal groups, one group to be exercised and the other to be confined. In dividing each litter attempts were made to distribute the pigs according to sex, weight, and vigor. All litters were confined indoors, and on or before the third day the exercise was begun. During the night all pigs were allowed to remain with their mothers. During the day all pigs were confined in barrels with straw as bedding. At stated intervals during the day all pigs were placed with their mothers to feed for short periods of time. The groups to be exercised were removed from the barrels and driven up and down the concrete runway of the farrowing house twice daily. The runway was about 55 feet long, and the pigs were driven up and down this runway 7 to 14 times each exercise period. There were 10 exercised pigs, 7 of which became anemic; there were also 10 pigs not exercised, and 8 of these became anemic. The experiment was discontinued after 4 weeks, during which time there were 2 deaths in each group, apparently unassociated with anemia because the deaths occurred during the first week.

During this exercise test the little pigs had little, if any, chance to consume any feed other than the sow's milk. While the pigs were with the sows during the day for short intervals, they were closely watched to see that they did not root around in either the sow's feed or feces. It was only after dark that the pigs were allowed to stay with their mothers for any length of time, and it is doubtful that they stirred around much during this time. As an additional precaution, all metal parts of the pens were wrapped with burlap.

The blood-hemoglobin concentration and live-weight curves for these pigs are shown in figure 6. The results of this test indicate that exercise, in itself, is not a factor either in the development or in the prevention of anemia in pigs. It is quite possible, however, that exercise may, under normal conditions of management, increase the appetites of the pigs to an extent not satisfied by the milk supply of the sow, and in this case they take to solid feed quicker than would otherwise be the case. This, as well as any other factor which would induce the pigs to consume feed, feces, soil, etc., at an early age, would certainly tend to prevent the development of anemia.

The onset of anemia⁷ in suckling pigs usually occurs between the third and fifth weeks, although anemia was often observed as early as the second week and may persist as long as the sixth week or even longer. The highest death rate occurs between the third and fifth weeks. Usually if the pigs live beyond the fifth week they have learned to eat solid feed and they recover rapidly. Average figures for live weights of pigs at 6 weeks of age seem to bear this out. The average live weight for all treated groups at 6 weeks of age was 19.5 pounds, and the average for the untreated pigs at the same age was also 19.5 pounds.

⁷ In this investigation a pig is considered anemic when the blood-hemoglobin concentration reaches a value of 3.5 g or less per 100 cc of blood.

It has been estimated that approximately 35 percent of all pigs farrowed in the United States die before they reach the weaning age. That anemia may be an important factor in this connection, especially in early litters, is strongly indicated by the results of this study. In the following calculations concerning the incidence of anemia and death, all pigs that died or were killed before the eighth day were excluded inasmuch as their deaths were, in all probability, unrelated to milk anemia.⁸

The data indicate that among pigs restricted to a diet of milk only and confined either indoors or outdoors on board or concrete floors, anemia is not only more prevalent but also the mortality is greater during February and March than during the summer and fall. Of the pigs in lot 1, inside, nontreated group, 100 percent of the February pigs became anemic, and 57 percent died; 100 percent of the March

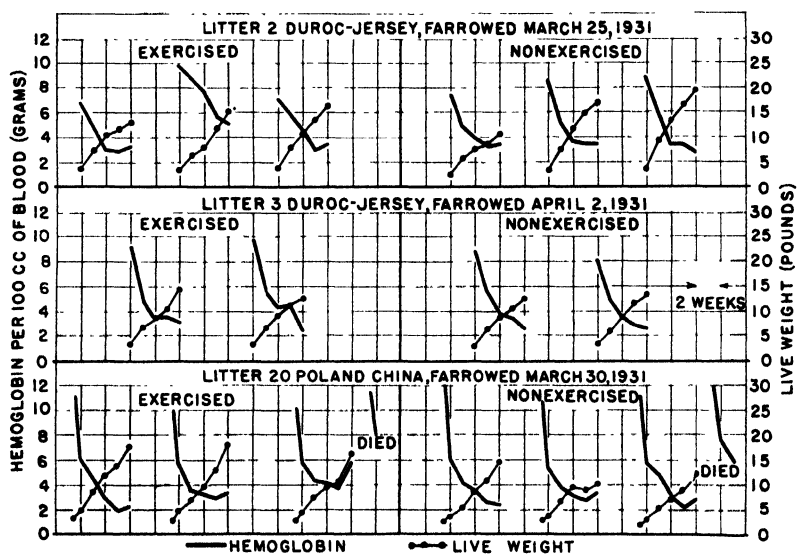


FIGURE 6—Blood-hemoglobin concentration and live weights of exercised and unexercised pigs which received no mineral supplement.

pigs became anemic, and 20 percent died; 58 percent of the April pigs became anemic, but none died; and none of the July pigs became anemic and none died. Of the pigs in lot 2, outside, nontreated group, 100 percent of the February, 100 percent of the March, 86 percent of the April, 20 percent of the September, and 100 percent of the October pigs became anemic. The deaths in this group were 88 percent of the February pigs only.

A comparison of lots 1 and 2 indicates (1) that anemia may be just as prevalent and the mortality just as high in the outdoor litters as in the indoor litters in the very early months, (2) that anemia may be quite readily produced in litters restricted to board and concrete floors out doors any time of the year, and (3) that, although anemia

⁸ As is customary in the university herd, pigs are vaccinated against cholera before they are weaned. All pigs in this investigation between the ages of 4 and 8 weeks were, therefore, on April 22, 1930, vaccinated with serum only and, on May 2, 1930, with the double treatment. Neither of these treatments seemed to affect the blood "picture" of the pigs, and there were no deaths following either treatment.

may be prevalent out doors on concrete, the mortality is not so great as indoors on concrete.

Grouping all treated pigs together, and all untreated (including lot 4) pigs together, it was found that of 157 pigs untreated, from February to October, 55 percent became anemic and 13 percent died, 24 percent of the anemic pigs died and none of the nonanemic pigs died. Of 38 treated pigs, farrowed between February and April, none became anemic. One pig in this group was missing. A closer examination of the untreated pigs shows that of 55 pigs farrowed in February and March, 96 percent became anemic and 38 percent died; 40 percent of the anemic pigs died, and none of the nonanemic pigs died.

As a practical aid in determining which of the several groups produced the largest numbers of satisfactory pigs, practically all the purebred litters of the 1930 spring season were placed, as previously described, in five groups and treated according to the procedure designated for that particular group. Some modifications in treatment were necessitated because of lack of barn space, and this modification amounted to the addition of two more groups, in one of which the udder treatment was followed by removal of the pigs to cindered lots not later than 2 weeks after farrowing, and in the other no treatment was given during the first 2 weeks and then the pigs were removed to cindered lots. The use of cindered pens was made because of the highly satisfactory results obtained during the previous year where this method was employed.

Sixty-two litters, a total of 301 pigs, were thus divided into 7 lots. The subsequent performance, especially in regard to the suitability for further feeding trials beginning in July and August, of all pigs available, was recorded. While practically all the pigs used in this investigation were eventually placed in feed-lot trials, the ones selected at this time were the best available. The selection of pigs for feed-lot trials was entirely unbiased, for after the 8 weeks of treatment all pigs were combined or mixed without regard to litters or previous groupings into convenient-sized pens for summer growth. It was not until after the feed-lot trials were started that the individual pigs were traced by their numbers to determine to which group in the anemia experiment they belonged. It was then found that 53 percent of the pigs in the treated groups were selected; 56 percent of the pigs which received no treatment but which were moved outside on cindered pens not later than 2 weeks of age were selected; while but 46 percent of the pigs that were untreated during all or the first 4 weeks of life were suitable at this time.

These data indicate that the treatments imposed to prevent anemia are in no way harmful to the subsequent health and well-being of the pig. Anemic pigs, although completely recovered from that condition, may be slower to regain complete health and proper appearance than pigs which have never had the disease. That subsequent growth of pigs once anemic may be interfered with is also indicated, although the average weights of pigs once anemic and of those which had never been anemic was found to be identical at 6 weeks of age.

As to the immediate cause of death of anemic pigs there is no direct evidence. It is perfectly obvious that as the anemic condition progresses the pig becomes weaker and weaker. Post-mortem examination shows a greatly enlarged heart -- obviously nature's attempt to

send a greater volume of the weakened blood to the tissues. The pleural cavity is also filled with fluid so that the overworked heart is working against unusual pressures. The mere act of laying a severely anemic pig across one's knee for the taking of blood samples or of running him a short distance is sufficient to cause him to collapse. It seems most likely that the lessened vitality of the anemic pig makes him more susceptible to respiratory infections and other diseases which may be the immediate cause of death.

STATISTICAL STUDIES

Since the percentage of cell volume and the hemoglobin concentration were determined in samples of blood taken from pigs soon after birth and at least weekly for a period of several weeks, ample data were available for the study of the correlations between these two measurements as well as those between either of these measurements and the hemoglobin saturation of the cells, the latter measurements being readily calculated from the former two.

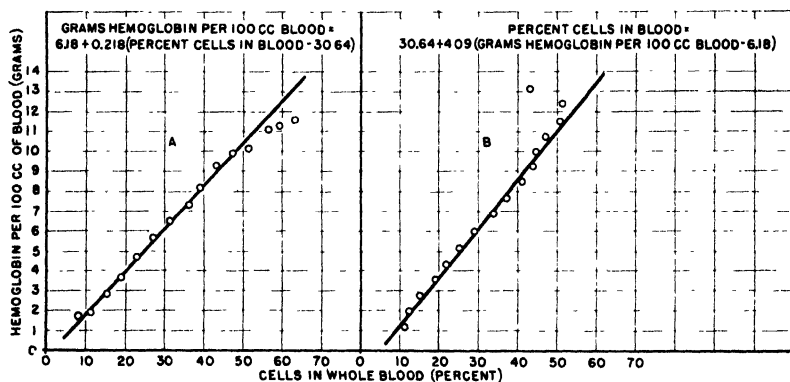


FIGURE 7 - Regressions of (A) hemoglobin concentration of blood on percentage of cells in the blood and (B) percentage of cells in the blood on the hemoglobin concentration of the blood

It was obvious from the first that a high degree of correlation existed between the hemoglobin concentration and the cell volume in the blood of anemic pigs. Statistically this relationship, as expressed by the correlation coefficient r , was found to be $\pm 0.945 \pm 0.0024$.

The regression equations calculated from these studies are:

- (1) Percentage cell volume = $30.64 + 4.09$ (hemoglobin concentration of blood - 6.18).
- (2) Hemoglobin concentration of blood = $6.18 + 0.218$ (percentage cell volume - 30.64).
- (3) Hemoglobin concentration of cells = $19.52 + 0.705$ (hemoglobin concentration of blood - 6.11).
- (4) Hemoglobin concentration of blood = $6.11 + 0.521$ (hemoglobin concentration of cells - 19.52).
- (5) Percentage cell volume = $30.47 + 1.342$ (hemoglobin concentration of cells - 19.54).
- (6) Hemoglobin concentration of cells = $19.54 + 0.0965$ (percentage cell volume - 30.47).

The regression lines are shown in figure 7. It may be readily seen that the plotted means of arrays cluster more closely around the regression lines below about 10 g of hemoglobin per 100 cc of blood than

they do above this value. This can hardly be explained on the basis of fewer data available in this upper region or of a greater biological variation when hemoglobin values exceed about 10 g per 100 cc of blood because of the fact that the means of arrays above this value all lie to one side of the regression line. The most probable explanation seems to be that, above a value of about 10 g of hemoglobin, there is little or no correlation between the blood-hemoglobin concentration and the cell volume. This would seem to indicate that above a certain hemoglobin concentration there may be an increase traceable to a hypersaturation of cells with little or no increase in cell volume.

When the hemoglobin concentration and the cell volume are known, it is a simple matter to calculate the hemoglobin concentration of the cells (expressed in grams of hemoglobin per 100 cc of cells). It was found that there was a significantly positive correlation between the hemoglobin concentration of the blood and the hemoglobin concentration of the cells. The correlation coefficient $r = +0.606 \pm 0.014$. This indicates that as the hemoglobin concentration of the blood decreases there is also a decrease in the hemoglobin saturation of the cells, and, since the erythrocytes make up such a large percentage of the cell volume, it means that there is a decrease in the saturation of the erythrocytes. This statement is supported by the calculations given in table 1. The figures in the last two columns of this table show that, as the blood-hemoglobin concentration decreases from 8.98 to 1 g per 100 cc, the concentration of hemoglobin in the erythrocytes decreases from about 14×10^{-12} g to about 11×10^{-12} g per erythrocyte. This decrease is small but consistent and is in harmony with the positive correlation mentioned above. It may be noted, however, that apparently there is no decrease in the mean corpuscular volume as the anemic condition becomes progressively worse. The mean corpuscular volume was found to be about $65\mu^3$. This is but slightly higher than the value of $57\mu^3$ reported by Götze (14) for the adult of the same species. The average hemoglobin content of the erythrocyte of adult swine blood has been reported by a number of workers (11, 14, 28, 36), and the values given range from 17 to 22×10^{-12} g of hemoglobin per erythrocyte. These values are slightly higher than the value of 14×10^{-12} g reported here for the new-born pig.

TABLE 1.—Size and hemoglobin content of erythrocytes at different stages of anemia

Age or condition	Hemoglobin per 100 cc blood	Cell volume	Erythrocytes per mm^3 ^a	Erythrocyte		
				Volume	Hemoglobin content	
					$\times 10^{-12}$ gram ^b	$\times 10^{-12}$ gram ^c
	Grams	Percent	Millions	Cubic microns		
Less than 30 hours old	8.98	44.1	6.74	65	14.0	13.6
Slightly anemic	4.60	23	3.48	66	12.2	12.4
Moderately anemic	3.85	21	3.31	63	11.4	11.8
Severely anemic	1.7	16	2.47	65	10.62	11.73
Extremely anemic	1	10	1.5	67	10.60	11.70

^a The number of erythrocytes in new-born pigs was taken from Hamilton, Hunt, Mitchell, and Carroll (15), while those values given for the anemic pigs were obtained in this study. The latter data were obtained on but few samples, so that they, as well as the calculations made from them, are to be considered more because of their relative rather than their absolute significance.

^b Calculated from regression equation: hemoglobin concentration of cells = $19.52 + 0.705$ (hemoglobin concentration of blood - 6.11).

^c Calculated from regression equation: hemoglobin concentration of cells = $19.54 + 0.0965$ (percent cell volume - 30.47).

A third correlation was possible in this connection, i.e., a correlation between hemoglobin concentration of the cells and the cell volume, and this was also significantly positive; $r = +0.360 \pm 0.020$.

It may be seen from the last two correlations that there is a closer correlation between hemoglobin saturation of the cells and blood-hemoglobin concentration than there is between hemoglobin saturation of cells and cell volume, the former correlation being, $r = +0.606 \pm 0.014$, and the latter, $r = +0.360 \pm 0.020$, the difference being 0.246, with a probable error of the difference of 0.024.

Thus the decrease in hemoglobin concentration of the blood, which occurs during the development of nutritional anemia, seems to be brought about not only by a decrease in cell volume but also, though not so important a factor, by a decrease in the hemoglobin concentration of the red cells.

A common opinion among practical swine raisers is that the incidence of anemia is greatest in litters that grow the most rapidly during the first few weeks. The data obtained in this study afforded an opportunity to test the validity of this opinion. The correlation between the decrease in blood-hemoglobin concentration occurring during the first 3 weeks and the increase in live weight during the same period was calculated for all untreated pigs in this investigation. Data were available for 72 pigs, and the correlation coefficient was found to be $+0.33 \pm 0.070$. Although not a high degree of correlation, it is nearly five times its probable error and may therefore be considered significant. The obvious explanation is that the more rapidly growing pig uses up his bodily store of minerals required for hemoglobin formation, which are present in insufficient concentrations in the milk, before it is old enough to consume solid food which contains the essential minerals, and therefore becomes more severely anemic than the slower growing pig.

THE NATURE OF THE INITIAL DECREASE IN BLOOD-HEMOGLOBIN CONCENTRATION AND CELL VOLUME

In previous publications from this laboratory (15, 20) attention has been called to the fact that the concentration of hemoglobin in the blood of pigs begins to decrease at or very soon after birth. By determining daily for the first 5 days the hemoglobin concentration in the blood of a few litters of pigs treated with iron and copper, it was found that the hemoglobin concentration decreases continuously for 3 or 4 days regardless of the presence of an abundance of hemoglobin-forming constituents in the diet. However, if the milk diet is supplemented with iron or with iron and copper salts the hemoglobin concentration begins to increase about the fifth day and continues to increase so that by the end of the third or fourth week of life the birth level has been equaled or even surpassed. In the case of the pig the blood-hemoglobin concentration at birth seems to be about the same as the adult level.

In this investigation the hemoglobin concentration and cell volume were determined in blood samples taken as soon after birth as was possible, again in samples taken on the third or fourth day, and then at weekly intervals thereafter. Since some of the pigs were restricted to a diet of milk alone and others received an iron, or iron and copper, supplement with their milk, it was possible to study the magnitude

of the initial decrease, as measured by the hemoglobin concentration in the blood on the third or fourth day, in the presence and absence of the mineral supplements. The magnitude of the initial decrease in hemoglobin was determined by expressing the hemoglobin concentration on the third or fourth day as a percent of that found at birth of the pig. These values were averaged for the two groups, and for 61 untreated pigs the average was 68.5 ± 9.8 , and for 28 pigs receiving the mineral supplement the average was 79.4 ± 6.8 . While the difference between the means is 10.9 percent in favor of the pigs that received the mineral supplement the probable error of the difference is ± 12 , showing the difference to be far from a significant one.

That the hemoglobin concentration in the blood of the treated pigs increases rapidly after the initial decrease and that it continues to decrease in rate on an unsupplemented milk diet is shown by the fact that the average hemoglobin concentration between the eighth and eleventh days for 16 treated pigs was 90.3 percent of their birth levels, while the corresponding average for 74 untreated pigs was 46.1 percent of their birth levels.

The cause of this initial decrease in blood-hemoglobin concentration is not explained by the data obtained in this investigation but, because of the consistency in the correlation between blood-hemoglobin concentration and cell volume in the blood from birth levels downward to severe anemic conditions, some speculation as to the nature of this initial decrease seems warranted.

The data presented above indicate that as the anemic condition develops in the pig, the hemoglobin concentration of the blood decreases not only because of a gradually decreasing number of red cells per unit of blood volume, but also because the hemoglobin concentration of the red cells also decreases. Insufficient data are available to show whether or not there is any change in the hemoglobin concentration of the cells coincident with the initial decrease in blood-hemoglobin concentration. Nevertheless it is certain that this initial decrease is caused mainly by a rapid decrease in the number of red cells per unit of blood volume.

A decrease in the number of red cells per unit of volume may be brought about either by a destruction of red cells more rapid than their formation or by an increase in blood volume in which the plasma is formed at a faster rate than are the red cells. It is conceivable that the formation of the blood cells during the first few days of postnatal life might be slower than it is after the animal has become acclimated to postnatal conditions, and it is also possible that there is some increase in red-cell destruction. That the latter may be a possibility of considerable importance may be inferred from studies made on the blood of new-born infants.

Rowntree, Brown, and Roth (31) have reviewed most of the work on the blood of the new-born infant prior to 1929. They conclude that the blood of infants at birth is extremely high in hemoglobin, often above 20 g per 100 cc of blood. More recent confirmation of this has been obtained by Börner (2) and by Elvehjem and Peterson (13). This high hemoglobin concentration is accompanied by a high cell volume. During the second week of the infant's life there is a decrease in hemoglobin concentration and cell volume, so that by the end of the second week these values have been reduced to those which

are considered normal for the adult. This decrease in hemoglobin concentration and cell volume is explained by Rowntree, Brown, and Roth (31) as being due to a hemolytic crisis which is probably related to icterus neonatorum. Whether or not this explanation applies to the initial decrease in hemoglobin concentration and cell volume that has been observed in the new-born pig, which has a hemoglobin concentration and cell volume equal to but no higher than that of the adult pig, remains to be shown.

SUMMARY

Nutritional or milk anemia in suckling pigs is more prevalent in February and March litters than in those farrowed in the late spring, summer, or fall. The disease is not only more prevalent during February and March, but it is also more severe and the death rate is greater. The disease, however, may be induced in litters farrowed during the late spring, summer, or fall by confinement of the litters indoors on board and concrete floors.

Anemia is just as prevalent in litters kept outdoors in individual houses with access to concrete runways as it is in litters confined indoors. Under outdoor conditions the mortality is somewhat less than under indoor conditions.

Brushing the udders of the sows with a dilute aqueous solution containing iron, copper, and sirup, or iron and sirup, or iron alone, one or more times daily from farrowing until weaning time, completely prevents anemia in suckling pigs. The udder treatment for the first 4 weeks followed by allowing the little pigs access to a palatable feed mixture containing 0.1 percent of added ferric sulphate is an alternative method which seems to be just as sufficient as is the udder treatment for the entire 8 weeks.

Of 55 pigs, farrowed during February and March and confined either indoors or outdoors on board and concrete floors without treatment, 96 percent became anemic and 40 percent of the anemic pigs died. Of 38 pigs, farrowed during February and March and confined indoors, that received either the iron and copper or iron treatment administered to the sows' udders, not one became anemic.

Nutritional or milk anemia is an important factor in the mortality of pigs before weaning time. Of 86 pigs having a blood-hemoglobin concentration of 3.5 g or less per 100 cc, 24 percent died. Of 109 pigs whose blood-hemoglobin concentration did not reach anemic levels, only 1 pig was lost; and whether or not this pig died, was eaten, or strayed away is not known.

Pigs receiving their mineral supplements through the udder treatment described above suffer no subsequent ill effects. A slightly greater percentage of treated pigs were suitable for feeding trials by July and August than was the case with untreated pigs.

Exercise in itself is not a factor in the prevention or the development of anemia.

The average hemoglobin concentration of 163 pigs less than 24 hours old was 8.98 g per 100 cc of blood, and the average cell volume of 98 pigs less than 24 hours old was 44.1 percent.

The correlation between the hemoglobin concentration in the blood and the cell volume is, $r = +0.945 \pm 0.0024$. The correlation between hemoglobin concentration in the blood and the hemoglobin saturation

of the cells is $r = +0.606 \pm 0.014$. The correlation between hemoglobin saturation of the cells and the cell volume is $r = +0.360 \pm 0.020$.

As the anemic condition becomes progressively worse, the blood-hemoglobin concentration, the cell volume, the number of cells, and the hemoglobin saturation of the cells decrease, while the corpuscular volume apparently does not change.

The mean corpuscular volume of the erythrocytes in new-born pig blood was found to be about $65\mu^3$ and the average hemoglobin content of an erythrocyte was 14×10^{-12} g.

Pigs which grow the fastest during the first 3 weeks are a little more likely to become anemic than are the more slowly growing ones. The correlation between the 3 weeks' increase in live weight and the 3 weeks' decrease in hemoglobin concentration was $r = +0.33 \pm 0.070$.

Pigs when farrowed have a hemoglobin concentration and a cell volume of about the same magnitude as those of the full-grown animal. During the first 3 or 4 days these values decrease to about two-thirds or three-fourths of the respective birth levels, regardless of the presence or absence of an abundant supply of hemoglobin-forming essentials in the diet. Possible causes of these initial decreases are suggested.

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ABSORPTION, UTILIZATION, AND RECOVERY OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY APPLE TREES GROWN IN CYLINDERS AND SUBJECTED TO DIFFERENTIAL TREATMENT WITH NUTRIENT SALTS¹

By WALTER THOMAS

Professor of phytochemistry, Pennsylvania Agricultural Experiment Station

INTRODUCTION

In a recent paper the writer (32)² presented data on the composition of the season's branch growth at successive periods of the growth cycle in relation to the vegetative and reproductive responses of apple trees subjected to treatment with different combinations of nutrient salts, when grown in metal cylinders. The present paper reports the total amounts of nitrogen, phosphorus, and potassium (as N, P₂O₅, and K₂O) absorbed during the whole 6-year growth period by a representative tree under each treatment.

The trees in each of the two culture systems—sod and tillage—in this experiment were exposed to identical external conditions of temperature, rainfall, light, and heat, and in addition to a uniform soil substrate (26). Considered, therefore, as separate systems, the only variables to which the trees under sod and tillage, respectively, were subjected, therefore, were the mineral nutrient salts added.

A voluminous literature on the absorption and utilization of the principal nutrient elements by plants is extant. The writer (27, 28, 29, 30, 31) has critically discussed and summarized our present knowledge of this subject with reference especially to the absorption of nitrogen, phosphorus, and potassium, and Hoagland (9, 10) has reviewed the more recent (1930-31) contributions. According to the views of Steward (24) and Hoagland (9) the process of absorption requires the expenditure of energy, the source of which is metabolic, determined by oxygen absorption or limited by carbon dioxide accumulation, the ultimate mechanism being determined by the electromotive forces brought about by oxidation-reduction potentials.

PREVIOUS INVESTIGATIONS ON THE TOTAL AMOUNTS OF NITROGEN, PHOSPHORUS, AND POTASSIUM REMOVED BY APPLE TREES

The object of the early experiments on the amounts of nitrogen, phosphorus, and potassium absorbed by the Pomaceae was to acquire information relating to the proximate amounts of these elements annually withdrawn from the soil by mature trees of maximum reproductive capacity.

Roberts (20) investigated a 13-year-old Wagener apple tree. The composition of the leaves sampled in the fall with respect to ash, nitrogen, phosphorus, and potassium was established. From these

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² Reference is made by number (*italic*) to Literature Cited, p. 579.

analyses and the average composition of apples obtained from another source, Roberts, by making numerous assumptions, presented an approximate estimate of the amounts of these nutrient elements removed by fruit and leaves at various ages of the tree.

Ten years later Van Slyke and his coworkers (34) reported the amounts of nitrogen, phosphorus, and potassium in the season's growth of a Baldwin and also of a Rhode Island Greening apple tree. The trees were about 30 years old. The approximate annual average removal of nutrients from the soil by trees of this age is reported as nitrogen, 1.5 pounds; phosphoric acid, 0.4 pounds; and potash, 1.6 pounds.

During the next decade Thompson (33) planted in the experimental plots of the Arkansas Agricultural Experiment Station seedlings obtained from five different known varieties of apple (average weight of trees 117 g). At the end of the growing season of each year the nitrogen, phosphorus, and potassium content of two trees of each variety were determined. Thompson reported the climatic conditions and also the analyses of the soil but did not mention either the fertilizer treatments (if any) or the culture system under which the trees were grown. Reference will be made later to certain of his data.

PLAN OF THE EXPERIMENT

Inasmuch as the plan of the present experiment has been given in detail in previous publications (1, 32), it will only be necessary to recapitulate briefly. In May 1922, stocks representing a single clone were obtained from the East Malling Research Station, England, and were planted in a Hagerstown silty clay loam soil. Two weeks later they were whip-grafted to Stayman Winesap scions from a tree whose performance was known for two-bud generations (1). Boiler-plate cylinders 5 feet in diameter and $5\frac{1}{2}$ feet deep were filled with the soil previously thoroughly mixed with respect to each of the three soil horizons (26, 32). Three years after planting, the trees (six in each row) were subjected to differential nutrient treatments by the application of various combinations of the following pure salts: NaNO_3 , $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, and K_2SO_4 . The combinations added were (1) $\text{NaNO}_3 + \text{CaH}_4(\text{PO}_4)_2 + \text{K}_2\text{SO}_4$ (symbol NPK); (2) $\text{NaNO}_3 + \text{CaH}_4(\text{PO}_4)_2$ (symbol NP); (3) $\text{NaNO}_3 + \text{K}_2\text{SO}_4$ (symbol NK); (4) $\text{CaH}_4(\text{PO}_4)_2 + \text{K}_2\text{SO}_4$ (symbol PK); (5) NaNO_3 (symbol N); (6) $\text{CaH}_4(\text{PO}_4)_2$ (symbol P); (7) one row of trees received no nutrient salt addition (symbol check).

Half of the trees under each treatment were maintained in a timothy and bluegrass sod, and the other half were subjected to a tillage system. The practical field details and also the growth records during the experiment period under each system have been given by Anthony and Clarke (1). In October 1927, a representative tree growing under (1) the sod, and (2) the tillage systems, respectively, in each of the nutrient salt treatments was removed and divided for purposes of chemical analyses into (1) leaves, (2) trunk, (3) large branches, (4) small branches, (5) large roots, (6) small roots, and (7) fruit (if any).

Representative samples of the portions so separated were secured for analysis by first cutting them into small pieces, and then reducing by the process of successive quartering used in ore sampling. The

final samples representative of each division were dried at 100° F. and then ground in a Wiley mill to pass a 100-mesh sieve. Because of the difficulty in removing all the attached soil by washing, the results for the roots are calculated to a silica-free basis. The analytical methods adopted were identical with those previously described (32).

EXPERIMENTAL DATA

The analytical results are summarized in table 1. The detailed analyses are omitted, but investigators desiring the detailed analyses of the grafted Stayman Winesaps and of the trees when dug up and also of the prunings may procure them from the research office of the School of Agriculture of this institution or from the author. Photographs of six of the trees grown in sod are given in figures 1 and 2. Photographs of some of the other trees appear in the paper by Anthony and Clarke (1).

TABLE 1.—*Total nitrogen, phosphorus, and potash (grams) removed by trees from cultivated and uncultivated soil, to which different mineral treatments were applied, during entire growth period, 1922-27*

EXCLUDING LEAVES

Treatment	Dry weight of trees (grams)		Total N absorbed during growth		Total P ₂ O ₅ absorbed during growth		Total K ₂ O absorbed during growth	
	Under cultivation	In sod	Under cultivation	In sod	Under cultivation	In sod	Under cultivation	In sod
Check	12, 228	8, 886	35 8	18 8	15 1	10 6	40 5	28. 7
NPK	14, 792	15, 265	136 8	142 8	26 1	26. 6	80 7	81 4
NP	16, 005	15, 007	129 6	138. 2	23 1	25. 2	71. 4	65. 3
NK	14, 504	15, 008	96. 5	96. 7	18 5	19. 9	69 0	76. 6
PK	8, 996	8, 326	39. 8	28. 6	13 0	13 4	41 5	33 8
N	12, 109	13, 053	83. 3	84 0	15 3	16. 4	51. 1	56 0
P	12, 239	7, 595	53 4	31 2	17 1	11. 5	50 1	29. 5

INCLUDING LEAVES

Check	14, 797	11, 433	53 5	35 4	17. 6	12 9	65 4	52 1
NPK	18, 365	18, 891	180. 9	187. 7	31. 6	32. 1	140. 4	136. 9
NP	19, 277	18, 975	170 3	180 3	27. 8	29 9	109 3	99 6
NK	17, 679	18, 430	131. 8	127 3	22. 6	23 5	114 4	113 4
PK	11, 788	11, 054	63. 4	55 0	15. 8	17. 3	79. 7	71 1
N	15, 419	16, 139	121. 3	119. 0	19. 9	20 7	90. 9	93 0
P	14, 936	9, 907	77. 1	47. 9	20 4	14. 0	79. 8	54. 9

INTERPRETATION AND DISCUSSION OF RESULTS

The distinguishing feature of the results is the great differences in the assimilation of nitrogen, phosphorus, and potassium resulting from the different nutrient salt treatments.

In considering the effect of these different treatments on carbohydrate metabolism, it has been found (32) that the concentration of carbohydrates in the season's growth follows the ascending series NPK < NP < NK < N < PK < P < check, showing that carbohydrates have accumulated in much larger amounts in the trees which did not receive nitrogen additions. The relatively small absolute amounts of nitrogen, phosphorus, and potassium found (table 1) in the trees receiving no nitrogen is an indication that the low concentrations of



FIGURE 1.—Trees grown in sod: A, no. E 1, treated with monocalcium phosphate only (symbol P); B, no. E 2, treated with sodium nitrate only (symbol N); C, no. E 3, treated with sodium nitrate, monocalcium phosphate, and potassium sulphate (symbol NPK).



FIGURE 2.—Trees grown in soil: A, no E 4, untreated check; B, no E 5, treated with monocalcium phosphate and sodium nitrate and monocalcium phosphate (symbol NP); C, no. D 7, treated with potassium sulphate (symbol PK).

these elements calculated earlier (32) on the basis of percentage of dry weight of the season's branch growth is the result of a much lower intake of these elements and not a masking effect caused by the relatively high amounts of carbohydrates.

The concentration of nitrogen in the season's growth has been shown to follow the ascending series check $< P < N < NK < PK < NPK$ (32). This order closely parallels the data for the total growth of the trees under each of the respective nutrient treatments. These results explain why the nitrogen factor plays such a dominant role in fruit growing.

SPECIFIC EFFECT OF EACH OF THE PRINCIPAL ELEMENTS

The writer has summarized (28, 29, 31) the results of field experiments which supply data on the effect produced, if one of the principal nutrient elements (nitrogen, phosphorus, or potassium) is omitted from a fertilizer. In some of these experiments (13) the omission of one of these elements resulted in an increased absorption of the other elements of the fertilizer with concomitant decrease in growth and yield of fruit attributed to a nutritional lack of balance. But in other field experiments (23, 31) the omission of an element from a fertilizer containing all three elements (nitrogen, phosphorus, and potassium) resulted in a decreased absorption of the other elements present. An explanation of such anomalous results has already been given by the writer (31).

The results summarized in table 1, which shows the amounts of nitrogen, phosphorus, and potassium (as N, P_2O_5 and K_2O) absorbed by the trees during the entire growth period of 6 years, indicate that the omission of one of these elements from a fertilizer has resulted in a decreased absorption and assimilation of the elements present in the incomplete fertilizer as compared with the complete fertilizer (cf. the amounts absorbed by the trees which received two nutrient elements, viz, NP, NK, and PK, with the absorption by the trees treated with all three nutrients, NPK). Such results are in accordance with Liebig's law of the minimum. This decreased absorption resulted in a lack of nutritional balance characterized by decreased growth and blossom formation except in the NP treatment, in which a decreased absorption of potassium resulted in slightly increased growth and blossoming.

In attempting to isolate the specific effect of an element in relation to the energy transformation in any living system, it is necessary to keep in mind the fact that the Wirkungswert (effect factor) of an element may not be (and generally is not) the same in the presence of another factor or factors as when the factor operates alone. Experimental results may be cited in which, when two factors vary simultaneously, each may produce its own effect independently of the other, as in Mitscherlich's experiments (16, 22). Mitscherlich found that when oats were grown in sand receiving varying amounts of water and calcium phosphate the relative effects on the yield produced by the different amounts of water were constant however much phosphate was added. But since numerous investigators (5, 7, 14, 19) have been unable to confirm the constancy of Mitscherlich's Wirkungswert for each fertilizer, the general principle enunciated in the opening of this paragraph must be accepted.

The data of the present experiment show that the amount of an added element absorbed by a plant and its effect on growth and reproduction is a function of the elements with which it is associated. The plant has a capacity to grow in a manner that cannot be regarded as a simple process of addition and subtraction of single effect factors. It is with these limitations in mind that the absorption and recovery of elements are discussed. An interesting and lucid explanation of the operation of growth factors is given by Nolte (17).

EFFECT OF THE OMISSION OF NITROGEN

Table 2 shows the amounts by which the absorption of each of the elements has been reduced by the omission of nitrogen and the quantitative effect of this reduced absorption in reducing growth and blossoming. The values given in columns 2 to 7 represent the difference in the amounts of the elements absorbed in each of two comparisons from which nitrogen has been omitted.

TABLE 2.—Effect of the decreased absorption^a of each of the nutrient mineral elements in reducing growth and blossoming following the omission of nitrogen, phosphorus, or potassium

Treatments compared	NITROGEN OMITTED									
	N		P ₂ O ₅		K ₂ O		Growth		Blossoms removed to thin to 6 inches apart, 1925-27	
	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod		
	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Number	Number
NPK and PK	117.6	132.7	15.7	14.7	60.6	55.7	6,577	7,836	530	727
NP and P	93.3	32.5	7.4	15.9	29.5	44.6	4,341	9,068	689	699
	PHOSPHORUS OMITTED									
	49.2	60.4	8.9	8.6	25.9	23.5	686	461	272	348
	48.9	61.5	7.8	9.3	18.5	3.6	2,858	2,836	679	576
	POTASSIUM OMITTED									
	10.7	7.3	3.8	3.2	31.0	37.3	^b 911	^b 84	^b 195	28
	10.4	8.2	2.7	2.8	34.6	58.4	2,261	2,291	212	256

^a Differences, in grams, in quantities of each element absorbed are shown in columns 2 to 7.

^b In these cases only, an increase and not a decrease occurred.

The data in table 2 indicate that no simple quantitative relationship exists between the amounts by which the absorption of each of the elements have been reduced as a result of the omission of nitrogen from the fertilizer and the accompanying reduction in growth and blossoming. The effect values of nitrogen applied together with phosphorus are not the same when potassium is present as when that element is absent. Moreover, this effect changes from a negative to a positive value in the trees under cultivation and sod, respectively.

EFFECT OF THE OMISSION OF PHOSPHORUS

Table 2 also shows the amounts by which each of the elements has been reduced as a result of the omission of phosphorus from the fertilizer and the accompanying disturbance in metabolism shown by the extent to which growth and blossoming have decreased. The values given in the table express the difference in the amounts of the elements absorbed in each of two comparisons from which phosphorus has been omitted.

The table is of interest because it shows that the quantitative effect of the omission of phosphorus on the reduction of growth and blossoming is much greater in the absence of potassium than when this element is present. In other words, the effect factor for phosphorus when applied together with nitrogen is much greater if no potassium is added. Since the differences in the amounts of nitrogen absorbed under the respective culture systems in both comparisons are practically identical, one must attribute the marked effect of the omission of phosphorus in the present experiments to differences in the amounts of potassium absorbed.

EFFECT OF THE OMISSION OF POTASSIUM

Table 2 further shows the quantitative effect of the omission of potassium on the reduction in the absorption of each of the elements and the effect of this reduced absorption on growth and blossoming.

The omission of potassium resulted in increased growth in the combination NP, as compared with NPK, both under cultivation and in sod, and also in increased blossoming under cultivation. An explanation of this anomalous behavior will be apparent later from the discussion (p. 576) of the ratios in which the elements have been absorbed.

UTILIZATION AND RECOVERY OF NITROGEN, PHOSPHORUS, AND POTASSIUM

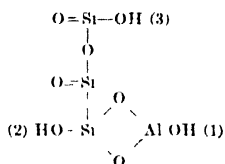
Theoretically a distinction must be drawn between the amount of an element absorbed and the amount utilized by the plant. Some authorities (11) claim that the absorbed but unutilized portion of a nutrient element is unchanged and is found as dissociated inorganic salts in the vessels and cell sap, and, although critical experiments on the subject have yet to be conducted, the fact that the results of many investigators (2, 23, 35) indicate that at least in some plants the portion of an element absorbed in excess is expelled via the roots shortly after blossoming time, would lend support to this view. If Jacob's views (11) are correct, absorption and the utilization of an element as determined by the analysis of a plant at maturity would be synonymous. Some of the results of this experiment, however, do not conform to this concept.

Present usage of the terms "absorption", "assimilation", "utilization", and "recovery" are somewhat confusing. In the present paper the term "recovery of an element" will be used to indicate that portion of an element applied as fertilizer which has been absorbed by the plant.

The amount of an element absorbed is determined, among other factors, not only by the species of plant and the availability of the fertilizer per se, but also by the indirect effects produced by the interaction of the fertilizer (or salt) with the components of the solid and liquid phases of the soil and also by interionic effects. These

effects, which may be very complex, are manifested in the so-called "phenomena of antagonism" and in the disturbance of equilibrium through displacement of bases in the soil colloidal complex by one or more of the elements in the fertilizer, and in the phenomenon of fixation by the iron and aluminum of the aluminosilicates as well as by the microorganisms—the so-called "biological absorption" (25). These effects were very marked in the present experiment.

In the clay soils of the type used in the present experiment the outer hydrophilic coatings of the clay particles dominate the soil system and, inasmuch as the majority of soils give rise to negative aluminosilicate ions, low results for the recovery by the plant of cations may in part be explained on the basis of adsorption. But the explanation of the low recovery of anions such as the phosphate ion by such a similar fixation is more difficult to explain, unless the explanation of some investigators (12) be accepted, of the existence of a Helmholtz triple layer, or of Clarens (3) of a clay structure represented by the constitutional formula



in which the basic hydroxyl group (1) attached to the aluminum ion can be replaced by acid radicals.

RECOVERY OF NITROGEN

For reasons already given the recovery of an element from an added fertilizer cannot be determined with exactness in field experiments. In a controlled experiment of the present type (each cultural system considered separately), in which the trees have been subjected to the same growth factors except the addition of mineral salts, the values may be calculated accurately enough for practical purposes.

The values which represent the amounts of the respective elements absorbed from the added fertilizers given in tables 3, 4, and 6 were obtained from the difference between the amount of the element absorbed by the respective check tree and that of the treated tree under examination. This method gives values for the recovery somewhat higher than those that would be obtained by using instead of the check tree the tree which has received all the elements except the one of which the recovery is to be determined. The values for the recovery in the tables may thus be said to be upper limits.

In grain and vegetable crops the recovery of added nitrogen by the plant averages about 50 percent (15, 18, 21), but no data appear to be available on the recovery of nitrogen by fruit trees. In solution cultures at very high concentrations (0.02 to 0.16 percent) (8), the absorption-coefficient for the nitrate ion is very high, reaching 100 percent in the higher concentrations. The relatively low recovery by the plant of added nitrates in field experiments may be attributed not only to the low absorption-coefficient resulting from the relatively dilute concentration of the soil solution but also to the losses by leaching, to processes rendering the nitrogen immobile, and also (especially under cultivation) to losses as gaseous nitrogen.

The total amount of sodium nitrate applied to each of the trees receiving nitrate was 2,824 g, equivalent to 464.5 g calculated as elemental nitrogen. This corresponds to an annual application of 154.8 g, or to 0.33 pound of nitrogen per tree. The current practice in the experimental orchards of this station is to apply 5 pounds of sodium nitrate, equivalent to 0.8 pound of nitrogen, to mature trees, much larger than those used in the cylinders.

Table 3 shows the total nitrogen absorbed, the amount absorbed from the sodium nitrate added, and the percentage of the latter recovered by the plant.

TABLE 3 --Total nitrogen absorbed by growing trees, and total grams and percentage of nitrogen absorbed from the added sodium nitrate

Treatment	Trees under cultivation			Trees in sod		
	Total N absorbed	N absorbed from added NaNO_3	Added N utilized	Total N absorbed	N absorbed from added NaNO_3	Added N utilized
	Grams	Grams	Percent	Grams	Grams	Percent
Check	53.5			35.4		
NPK	180.9	127.4	27.4	187.7	152.3	32.5
NP	170.2	116.7	25.1	180.3	144.9	31.2
NK	131.7	78.2	16.8	127.3	91.9	19.8
N	121.3	67.8	14.6	119.0	83.6	18.0

The utilization and recovery of added nitrogen follows the descending series $\text{NPK} > \text{NP} > \text{NK} > \text{N}$. The addition of monocalcium phosphate and potassium sulphate to the sodium nitrate either singly or together has, therefore, increased the efficiency of the latter. The data also show that the addition of monocalcium phosphate has increased the absorption of soil nitrogen, inasmuch as the increase in the amount of nitrogen absorbed by the trees treated with monocalcium phosphate compared with the check trees is 23.6 and 12.5 g in the cultivation and sod systems, respectively. These results may be compared with those of the Rothamsted wheat plots, in which the absorption of nitrogen by the crops on plots treated with potash and phosphates is very much greater than on the plots which received no such additions (22).

RECOVERY OF PHOSPHORUS

In high calcareous soils the addition of monocalcium phosphate results in the formation of insoluble di- and tri-calcium phosphates, the formation of the latter being greater the greater the $\text{Ca}:\text{P}_2\text{O}_5$ ratio. In the presence of magnesium carbonate, similarly difficultly soluble, calcium and magnesium phosphates are formed. Gerlach's experiments (4) may be given as an illustration of the high fixing capacities of calcium carbonate, magnesium carbonate, aluminum hydroxide, and ferric hydroxide for the phosphate ion. The phosphorus held by calcium carbonate and magnesium carbonate, however, is readily dissolved by a saturated solution of carbonic acid, whereas none of the phosphate fixed by the sesquioxides was dissolved.

In the absence of calcium carbonate, superphosphate is supposed to react with iron and aluminum compounds to form basic dicalcic aluminum and iron phosphates. This fact explains why the soil solution and the sap of plants contain so little phosphorus following

fertilization with phosphates. The concentration of phosphorus in the soil solution ranges between 1 and 2 parts per million. The drainage water of soils contains only a trace of phosphorus even in sandy soils (6).

The total amount of $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ supplied to each of the trees that received phosphorus was 1,869.0 g, equivalent to 1,052.5 g phosphorus, and corresponding to an annual application of 350.8 g or 0.77 pounds of phosphorus per tree. The current practice in the experimental orchards of this station is to apply 250 pounds of 16 percent superphosphate per acre of 100 trees, corresponding to 0.45 pounds of phosphorus per tree.

The total amount of phosphorus absorbed, the approximate amount of added phosphorus absorbed from the added phosphate, and the percentage utilized (recovered) is given in table 4.

TABLE 4.—Total phosphorus absorbed by growing trees, and total and percentage of phosphorus absorbed from the added monocalcium phosphate

Treatment	Trees under cultivation			Trees in sod		
	Total P_2O_5 absorbed	P_2O_5 absorbed from added $\text{CaH}_4(\text{PO}_4)_2$	Added P_2O_5 utilized	Total P_2O_5 absorbed	P_2O_5 absorbed from added $\text{CaH}_4(\text{PO}_4)_2$	Added P_2O_5 utilized
	Grams	Grams	Percent	Grams	Grams	Percent
Check	17.6			12.8		
NPK	31.6	13.9	1.32	32.0	19.2	1.85
NP	27.7	10.1	.95	29.9	17.1	1.62
PK	15.8	-1.8		17.3	4.5	.42
P	20.3	2.6	.25	14.0	1.2	.17

The order of utilization and recovery of phosphorus in relation to treatment is $\text{NPK} > \text{NP} > \text{PK} > \text{P}$ except in the PK treated trees under cultivation, in which a negative absorption of added phosphate occurred. The utilization of phosphorus and the recovery of added phosphates by all the trees are very low, the range being from 0.12 to 1.85 percent. This may be compared with the 6.0 percent average recovery of phosphorus from superphosphates in the Rothamsted experiments from field crops (21).

Table 5 shows that the total quantity of phosphorus in the soil used in this experiment is quite small. The sesquioxides are high (aluminum oxide and ferric oxide equal 13.94 percent in the surface soil and 19.16 percent in the subsurface soil), as is also the calcium oxide.

TABLE 5.—Composition (percent) of the soil with respect to its principal basic and acidic constituents

Constituent	Surface soil to 9.5 inches	Subsurface soil, 9.5 to 18.5 inches	Subsoil, 18.5 to 42 inches	Constituent	Surface soil to 9.5 inches	Subsurface soil, 9.5 to 18.5 inches	Subsoil, 18.5 to 42 inches
SiO_2	72.330	67.670	63.115	CaO	0.680	0.655	0.395
Al_2O_3	10.257	13.585	15.984	MgO	.665	1.015	1.300
Fe_2O_3	3.690	5.580	6.840	K_2O	3.925	4.200	4.410
P_2O_5	.098	.100	.077	Na_2O	2.260	1.290	1.315

These high values for aluminum and iron sesquioxides may account for the exceptionally small recovery of phosphorus by the plants in this experiment. It should, however, be emphasized that the mecha-

nism by which phosphorus is rendered immobile in the soil has not been definitely established, and no advances have been made since the writer reviewed the subject (31).

RECOVERY OF POTASSIUM

The total amount of potassium sulphate applied to each of the potash-treated trees was 1,027 g, equivalent to 548.4 g of potash. This corresponds to an annual application of 182.8 g, or to 0.40 pound per tree. Table 6 shows the total amount of potash absorbed, the approximate amount of potash absorbed from the potassium sulphate added, and the percentage of the latter recovered by the plant.

TABLE 6.—Total potash absorbed by growing trees, total and percentage of potash absorbed from the added potassium sulphate

Treatment	Trees under cultivation			Trees in sod		
	Total K ₂ O absorbed	K ₂ O absorbed from added K ₂ SO ₄	Added K ₂ O utilized	Total K ₂ O absorbed	K ₂ O absorbed from added K ₂ SO ₄	Added K ₂ O utilized
	Grams	Grams	Percent	Grams	Grams	Percent
Check.....	65.4			52.1		
NPK.....	140.3	74.8	13.5	136.8	47.2	8.6
NK.....	114.4	49.0	8.9	113.3	61.2	11.1
PK.....	79.7	14.1	2.3	71.1	19.0	3.4

The order of utilization by the trees of added potash follows the descending series NPK > NK > PK. The amount of added potash recovered is relatively low. The range is from 2.3–13.5 percent. In field crops the average recovery of added potassium is 50 percent. The marked influence of sodium nitrate in combination with mono-calcium phosphate and also of sodium nitrate applied singly in increasing the absorption of potassium is apparent. This effect may be attributed to the influence of the sodium and calcium ions of the added salts on the replaceable bases and also to other factors discussed by the writer (31).

RELATIVE PROPORTIONS IN WHICH NITROGEN, PHOSPHORUS, AND POTASSIUM HAVE BEEN UTILIZED

The ratios of N:P₂O₅:K₂O calculated on the basis of phosphoric acid as the unit, are shown in table 7.

In Thompson's experiments (33) the ratio for trees which had the same approximate dry-weight production but which apparently received no fertilizers is 3.9:1:3.4.

An unsolved problem of great practical significance to agronomists and horticulturists is the question as to whether each crop has its specific N : P₂O₅ : K₂O ratio requirement, assuming that soil and climatic factors are constant. The results in table 7 lead to the conclusion that the ratios in which these elements are absorbed diverge from one another to a relatively small degree when plants that are optimum with respect to growth and reproduction are compared. In the present experiments, these are the trees which received the NPK and NP treatments.

TABLE 7.—*Variation in the ratio of nitrogen, phosphorus, and potash in the trees from cultivated and uncultivated soil to which different mineral treatments were applied*

Treatment	Soil under cultivation			Soil in sod		
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O
Check.	2.37	1	2.68	1.76	1	2.69
NPK.	5.21	1	3.09	5.37	1	3.06
NP.	5.59	1	3.08	5.49	1	2.59
NK.	5.20	1	3.72	4.85	1	3.85
PK.	3.05	1	3.18	2.13	1	2.51
N.	5.44	1	3.34	5.11	1	3.44
P.	3.11	1	2.93	2.72	1	2.57

INCLUDING LEAVES						
Check.	3.03	1	3.71	2.74	1	4.05
NPK.	5.72	1	4.44	5.85	1	1.27
NP.	6.13	1	3.93	6.02	1	3.33
NK.	5.82	1	5.05	5.42	1	4.83
PK.	4.00	1	5.04	3.17	1	4.10
N.	6.08	1	4.55	5.76	1	1.50
P.	3.78	1	3.92	3.41	1	3.92

These results serve to explain the apparently anomalous behavior of the trees which received no potassium, referred to on page 572, in which a relative decrease in the absorption of nitrogen and phosphorus resulted in increased growth under both sod and tillage and in increased blossoming under cultivation. It would appear that there is a very delicate balance between the ratio of these dominant nutrient elements for optimum growth and that the concentration of potassium is too high relative to the nitrogen and phosphorus in the trees which received potassium additions. The potassium concentration exerts an enormous influence in determining the rate of diffusion of carbon dioxide into the cell and up to the chloroplast surface.

COMPARISON OF THE RATIOS OF N·P₂O₅·K₂O ABSORBED BY TREES OPTIMUM WITH RESPECT TO GROWTH AND REPRODUCTION, WITH THE RATIOS IN WHICH THESE ELEMENTS HAVE BEEN APPLIED IN THE FERTILIZER ADDED

The method adopted in ascertaining the amounts of the respective elements which the trees under examination have absorbed from the respective salts added as fertilizer is not a mathematically rigorous one. This is a consequence of variation in the effect factors discussed on pages 570 and 572. The method of calculation is, however, sufficiently exact for all practical purposes. The very wide discrepancy between the ratio in which these elements are absorbed by the trees showing optimum physiological balance and the ratio in which the nutrient salts were applied is clearly brought out. The discrepancy is in part due to the soil properties, viz, leaching of nitrates and fixation of phosphorus and potassium.

After quantitative nature of these effects has been determined by an examination of the soil before and after the experiment, the theoretical economic quantities and ratios for optimum growth and reproduction of trees of this age will be given.

The total amounts of nitrogen, phosphoric acid, and potash added per tree were 464.5:1,052.5:548.4, equivalent approximately to a 3:8:4 fertilizer. In the trees analyzed the optimum ratio in which nitrogen, phosphoric acid, and potash have been absorbed is 175.6:29.7:124.8. By deducting from these values the amounts of nitrogen, phosphoric acid, and potash absorbed by the check trees, we get 122.1:12.1:59.5, or approximately 3.0:0.3:1.5 as the ratio in which these elements have been absorbed from the salts added compared with a 3:8:4 ratio actually applied.

An effort has been made and is still being made to remove fertilizer practice from the realm of empiricism. These results show the difficulties that must be overcome. The analysis of the plant alone is evidently insufficient. A knowledge of the fixing capacity of the soil is obviously of great importance. It follows that the application of the time-absorption-graph method on the entire plant (31) to the determination of the fertilizer requirements of the species when grown on soils of high fixing power necessitates a knowledge of the fixing capacity.

Although Wolff (36) provided definite proof that the composition of the ash of plants varied with the amounts and relative proportion of salts present in the nutrient culture or added to the soil, the present as well as the earlier results (32) cannot fail to emphasize anew the enormous differences that may be produced with respect to the percentage and absolute amounts as well as in the ratio of protein, carbohydrates, and ash constituents as the result of different fertilizer practices.

PRODUCTION OF DRY MATTER PER UNIT OF NITROGEN, PHOSPHORIC ACID, AND POTASH ABSORBED BY THE PLANT

Table 8 shows the amount of growth expressed in terms of dry weight of material formed per gram of the nutrient elements (as N, P_2O_5 , and K_2O) absorbed by the trees in each treatment. The data exhibit another form of the operation of the law of diminishing returns inasmuch as the dry weights produced per unit of nutrient absorbed in the various treatments decrease as the total amount of that nutrient absorbed increases.

TABLE 10.—Quantity of dry matter (grams) produced by the absorption of 1 gram of nutrient from cultivated and uncultivated soils to which different mineral treatments were applied

Treatment	Grams of dry matter produced per gram of indicated nutrient element applied to soil					
	N		P_2O_5		K_2O	
	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod	Soil under cultivation	Soil in sod
Check	276	323	840	888	226	219
NPK	101	100	581	589	130	138
NP	113	105	694	634	176	190
NK	134	145	780	785	155	162
PK	186	200	745	637	148	155
N	127	135	773	781	170	173
P	194	207	733	706	187	180

SUMMARY AND CONCLUSIONS

In May 1922, Stayman Winesap apple trees propagated by whip-grafting scions from a single parent tree on roots vegetatively propagated from a single tree were planted in a Hagerstown clay loam soil contained in 5- by 5.5-foot cylinders. Each year from 1925 to 1927, inclusive, applications were made of the pure salts sodium nitrate, monocalcium phosphate, and potassium sulphate in different combinations. The annual application per tree was equivalent to 0.33 pound of nitrogen, 0.77 pound of phosphoric acid, and 0.40 pound of potash. With respect to cultural systems half the trees were in tillage and half in sod.

In October 1927, an entire tree representative of each nutrient treatment and also of each cultural system was removed, and the amounts of nitrogen, phosphorus, and potassium assimilated during the growth period of 6 years were determined.

The percentage and absolute (total) amounts of each of the elements absorbed by the trees during the whole period of growth and also the ratio in which they were absorbed varied greatly with the nutrient treatments and also, but to a much lesser extent, with the cultural system.

The values for the total amounts of nitrogen, phosphorus, and potassium absorbed by an entire tree in each of the respective treatments indicate that the omission of any one of these nutrient elements from the complete fertilizer (NPK) is followed by a decreased absorption of the remaining elements. This decreased absorption resulted in a nutritional lack of balance as exhibited in reduced growth and flowering except in one case in which potassium was omitted.

The Wirkungswert (the effect factor) for nitrogen, phosphorus, and potassium, varied with the elements with which the element considered was associated in the added fertilizers. These effect factors are discussed in detail.

The percentage recovery by the trees of added nitrogen, phosphorus, and potassium is relatively low under all treatments.

There appears to exist a specific ratio in which nitrogen, phosphorus, and potassium are absorbed by the trees (NPK and NP) which are optimum with respect to growth and reproduction. This ratio is approximately 6:1:4.

The wide discrepancy shown between the ratio in which these principal nutrient elements are absorbed by the trees—even by those showing optimum physiological balance (NPK and NP)—and the ratio in which the nutrient salts were applied is indicative of soil effects, namely, leaching of nitrates, and of high fixing capacities of phosphorus and potassium.

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A FACTOR IN THE VARIETAL RESISTANCE OF CRANBERRIES TO THE FALSE-BLOSSOM DISEASE¹

By R. B. WILCOX, associate pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and C. S. BECKWITH, associate entomologist in cranberry and blueberry investigations, Department of Entomology, New Jersey Agricultural Experiment Station²

INTRODUCTION

Varieties of the cranberry (*Vaccinium macrocarpon* Ait.) under commercial culture differ widely in their susceptibility to the false-blossom disease. Fracker (5)³ was probably the first to mention this fact in a publication; he states that in the inspection of Wisconsin bogs for this disease, in the course of regulatory work, a few varieties had "practically clear records". Stevens (7), summarizing present knowledge of varietal susceptibility, includes the Howes and Centennial among the sorts that are very susceptible to false blossom, and says that the Early Black shows a fair degree of resistance under varied field conditions and the McFarlin shows marked resistance. Beckwith (2) classifies the Centennial and Champion as "only slightly less susceptible" to false blossom than the Howes. The evidence upon which to judge the Champion is less conclusive than that relating to the other sorts; this variety, in New Jersey, is not so generally affected as either Howes or Centennial, but it has become badly diseased in some instances when exposed to heavy infection. On the basis of field observations it may therefore be said that the five varieties mentioned fall into the following descending order of susceptibility to false blossom: Howes and Centennial, Champion, Early Black, and McFarlin.

Varietal resistance to false blossom is almost certain to play a large part in the future of the cranberry industry, especially in the development of new commercial forms. Up to the present time the only method for judging the resistance of a variety has been to watch its behavior under exposure to natural infection for several years. It has not been known whether the resistance of McFarlin is due to natural protection against attacks of the insect vector⁴ or to inhibition of the spread and survival of the false-blossom virus within the plant

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² The experiments reported herein were planned by the senior writer as one step in his attempt to devise a quick and reliable method of testing cranberry varieties for resistance to the false-blossom disease. They were conducted with the assistance of T. J. Blisard, formerly field assistant, New Jersey Agricultural Experiment Station, to whom acknowledgment is made.

³ Reference is made by number (italic) to Literature Cited, p. 590.

⁴ So far as known, false blossom is transmitted only by the blunt-nosed leaf hopper, as reported by Dobrosky (3). In the latest revision of the genus, Ball (1) gives this insect the name *Ophiola striatula* Fall. Common usage, however, still retains the name *Euscelia striatulus*.

tissues; or whether this variety may be a symptomless carrier of the disease.

There is no accumulation of evidence based on insect collections to indicate whether the blunt-nosed leaf hopper is relatively more abundant on Howes and Centennial bogs than on those of McFarlin or Early Black. In New Jersey, as a result of both natural conditions and cultural practices, such as the use of flood water for frost protection in June, the leaf-hopper population often varies greatly on plants of one variety, in a single bog, and even within the space of a few feet. Comparison between varieties in the field, therefore, is frequently misleading. A preliminary test showed little to be gained through casual observation of caged insects.

The present paper is a report of more careful experiments to determine whether the false-blossom vector prefers to feed on the "susceptible" varieties of cranberries rather than on those which are considered resistant; in other words, whether unattractiveness to the insect is a factor in the resistance of certain varieties. These experiments form part of an attempt by the senior writer to find a quicker and more reliable means of testing varietal resistance.

METHODS

Observation cages were prepared in the following manner: A 6-inch flowerpot was filled with moist sand and "planted" with 3 healthy uprights (cuttings) of each of the five varieties of cranberries heretofore mentioned, making 15 uprights in all. The pot was marked on the outside to identify the varieties. The uprights were enclosed in a lantern globe, the top of which was covered with cheesecloth. In this cage a number of mature leaf hoppers were placed. The interior of the cage could be examined from the outside without disturbing the insects. By keeping the sand moist, the uprights were maintained in good condition throughout the experiment (fig. 1).

On July 31, 1930, seven cages were prepared for the first series, the uprights for which were taken from bogs near Pemberton, N.J. About 25 leaf hoppers were introduced into each cage, and the cages were placed where they would receive direct sunlight only in the early morning and diffused light during the rest of the day. Observations were made in the afternoon. After the number of insects seen on cuttings of each variety had been recorded, each cage was turned so as to expose another variety to the strongest illumination for the next period. The experiment was continued until all the cages had been examined 10 or 11 times, making a total of 75 observations taken and 912 insect positions recorded.

A second series of 10 cages was prepared, the cranberry uprights for which represented the same varieties as before but were taken from bogs near Toms River, N.J. This series was not started until August 26, a very late date for work with this leaf hopper. The insect mortality was high, and fresh insects had to be added frequently. By this time, moreover, the cranberry fruit of the early varieties was very nearly mature. As it seemed possible that in the previous series the relatively large number of insects per cage might have interfered with their free choice of food plants; only five leaf hoppers were placed in each globe for the second trial. The cages

were kept just inside the screen of the laboratory porch at Toms River, where they received somewhat reduced sunlight during most of a clear day. They were examined once or twice daily until 18



FIGURE 1 —Type of observation cage used in experiments on varietal resistance of cranberries to the false-blossom disease.

observations per cage had been recorded, or a total of 180 observations. The number of insect positions observed, however, was only 574, less than two thirds of the number obtained from the first series.

RESULTS

The results of the first experiment, as summarized by cages, are given in table 1. It is apparent that about twice as many insects were found on uprights of the Howes as on those of the McFarlin variety, that the proportion on Centennial was also high, and that Champion and Early Black were in an intermediate position.

TABLE 1.—*Leaf hoppers observed on uprights of 5 cranberry varieties in each of 7 cages*

[Data represent summations of 11 readings unless otherwise noted]

Cage no	Leaf hoppers observed on uprights of -					
	Howes	Centennial	Champion	Early Black	McFarlin	All varieties
	Number	Number	Number	Number	Number	Number
1	32	33	42	29	17	153
2	36	35	20	25	26	142
3	30	48	22	39	19	158
4	36	24	24	28	16	128
5	37	23	23	24	4	111
6 ^a	36	30	22	15	19	122
7 ^a	27	20	23	10	18	98
Total	234	213	176	170	119	912
Percentage	25.7	23.4	19.3	18.6	13.0	100.0

^a 10 readings

In order to estimate the significance of these differences, the standard errors (standard deviations) were calculated. The variations were of the discontinuous type, and formed an asymmetrical curve. It was found by test that for each variety, and for the experiment as a whole, the frequencies with which 0, 1, 2, ... 9 insects were observed on a variety in a cage at one reading agreed satisfactorily with the frequencies calculated for a Poisson series according to the formula

$$e^{-m} \left(1, m, \frac{m^2}{2!}, \dots, \frac{m^9}{9!} \right),$$

in which m is the mean for the series, and $9!$ is "factorial 9".⁵ The correspondence between observed and calculated frequencies is shown in table 2. The last line gives for each series the value of P as obtained from the table of χ^2 distribution.⁶ This value represents the proportion of cases in which deviations from the calculated series will exceed the observed deviations. As agreement becomes closer, P approaches unity. The value $P = 0.05$ is regarded as the minimum to be accepted as indicating that the hypothesis accounts for the facts. Fisher states, "If P is between 0.1 and 0.9 there is certainly no reason to suspect the hypothesis tested."

⁵ For a discussion of the Poisson series and its characteristics, and of the χ^2 method of testing goodness of fit, see Fisher (4).

⁶ The value so obtained for the Early Black variety is somewhat too high; the necessary grouping of pairs for five or more insects in determining χ^2 disregards an obvious discrepancy in this case.

TABLE 2.—*Frequency of occurrence of various numbers of leaf hoppers on uprights of each of 5 varieties of cranberries, as observed and as expected according to Poisson distributions with similar means*

Number of leaf hoppers	Frequency of occurrence of leaf hoppers on uprights of—											
	Howes		Centennial		Champion		Early Black		McFarlin		All varieties	
	Ob- served	Ex- pected	Ob- served	Ex- pected	Ob- served	Ex- pected	Ob- served	Ex- pected	Ob- served	Ex- pected	Ob- served	Ex- pected
0	5	3.33	4	4.38	8	7.18	9	7.77	19	15.34	45	32.94
1	10	10.41	11	12.44	18	16.84	16	17.63	23	24.34	78	80.12
2	11	16.24	23	17.67	17	19.76	22	19.98	14	19.32	87	97.42
3	17	16.89	14	16.73	17	15.46	14	15.09	12	10.22	74	78.98
4	17	13.17	10	11.88	7	9.07	9	8.55	4	4.05	47	48.03
5	9	7.67	7	6.75	5	4.26	3	3.88	2	1.29	26	23.36
6	4	1.27	4	3.19	2	1.66	0	1.46	1	.34	11	9.47
7	2	1.91	1	1.30	0	.56	0	.47	0	.08	3	3.29
8	0	.74	1	.46	1	.16	1	.13	0	.02	3	1.00
9	0	.26	0	.15	0	.04	1	.03	0		1	.27
10 or more	0	.11	0	.05	0	.01	0	.01	0	---	0	.12
Total	75	75.00	75	75.00	75	75.00	75	75.00	75	75.00	375	375.00
<i>P^a</i>	0.64		0.75		0.92		0.97		0.56		0.30	

^a For explanation see p. 586

The variance of a Poisson series is definitely preestablished. Since the observations for each variety agreed reasonably well with this formula, the standard error of random sampling for a variety (based on total insects rather than the mean) could be represented by the square root of the number of insects recorded on that variety in all observations. For example, the total for Howes would be $234 \pm \sqrt{234}$. The standard error of a difference between two varieties ($a - b$) would then be $\sqrt{a + b}$; or, as an approximate figure to apply to a difference between any two of the five varieties, $\sqrt{\frac{2}{5}t}$, in which t is the total number of insect positions recorded on all varieties. Therefore, the very short formula used in these experiments for determining the standard error of a difference between the totals of two varieties was

$$\text{S.E.D.} = \sqrt{0.4t}.$$

This formula as applied to the first experiment gave a value of approximately 19, or 2.1 percent of the total number of insect positions. A difference exceeding twice this figure, or 38, was considered significant; a smaller difference might well be due to chance. In table 3 the differences between varieties are arranged in order of magnitude.

TABLE 3.—*Numerical differences between total number of insects found on uprights of 5 varieties of cranberries*

[Odds of probability calculated independently by Student's method]

Varieties		Difference in total number of leaf hoppers	Odds (Student's method)
More attractive to leaf hoppers	Less attractive to leaf hoppers		
Howes	McFarlin	115	1,924:1
Centennial	do	94	570:1
Howes	Early Black	64	78:1
do	Champion	58	76:1
Champion	McFarlin	57	43:1
Early Black	do	51	25:1
Centennial	Early Black	43	72:1
do	Champion	37	12:1
Howes	Centennial	21	6:1
Champion	Early Black	6	3:1

As a further test of significance the differences between all pairs of varieties were determined by Student's method from the values for each cage in table 1 taken as units. The odds determined from Love's modification of Student's table (6) were doubled, since, a priori, either variety of a pair might have been the more attractive.⁷ These odds, which express the probability against the differences being due to chance alone, are given in the last column of table 3. Odds of 22:1, or greater, are considered significant.

It will be seen that the independent use of these two measures of significance gave the following identical indications: The differences between the attractive Howes and Centennial and the unattractive McFarlin were highly significant; there was a significant distinction between any two varieties separated by two or more places in table 1, i.e., between Howes and Early Black or between Centennial and McFarlin; whereas there were only insignificant differences between the adjacent Howes and Centennial, Centennial and Champion, and Champion and Early Black.

Table 4 gives the results of the second series of cages, each value being the summation of 18 readings. Detailed frequencies for this experiment are omitted, but they showed satisfactory agreement with Poisson series. The standard error of a difference was approximately 15, or 2.6 percent of the total number of insect positions. The only differences exceeding twice these values were between Howes on the one hand and the four other varieties on the other hand.

TABLE 4.—*Leaf hoppers observed on uprights of 5 cranberry varieties in each of 10 cages*

[Data represent summations of 18 readings (second series)]

Cage no.	Leaf hoppers observed on uprights of—					
	Howes	Centennial	Champion	Early Black	McFarlin	All varieties
	Number	Number	Number	Number	Number	Number
1.	13	9	11	13	10	56
2.	13	5	7	14	7	46
3.	20	8	3	9	1	41
4.	9	9	17	5	11	51
5.	22	17	5	3	3	50
6.	9	11	18	13	11	62
7.	13	26	17	3	9	68
8.	12	11	13	5	13	54
9.	24	11	11	22	22	90
10.	16	8	10	9	13	56
Total	151	115	112	96	100	574
Percentage	26.3	20.1	19.5	16.7	17.4	100.0

DISCUSSION

The significant differences in the second series corroborated the results of the first series. The variation in the behavior of varieties in the two series was apparently connected with the maturity of the early varieties by the end of August. The fruit of Early Black and Centennial was practically mature; it might be expected that the foliage of these varieties would also have become much less active

⁷ For discussion see Fisher (4, p. 47).

than that of the late-maturing Howes and McFarlin,⁸ and for that reason might have been less attractive to the leaf hoppers. It is interesting to note, therefore, that the percentage of insects on Centennial and Early Black dropped two or three points in the second series, and that these losses were absorbed by the relatively immature Howes and McFarlin, while the midseason Champion remained stationary. This behavior emphasizes the delicacy of the test and the fact that cuttings of equal vigor and stage of maturity must be used if results are to be regarded with confidence. So far as could be detected, the smaller number of leaf hoppers per cage in the second experiment did not influence the feeding preferences of these insects.

As previously stated, results of the first experiment indicate that the Howes and Centennial were definitely the most attractive to the leaf hoppers and that the McFarlin was the least attractive. The percentages in table 1, however, should be considered as relative rather than as quantitative measurements of the attractiveness of the varieties.

If attractiveness be regarded as susceptibility to insect attack, the Howes was the most susceptible; the Centennial, slightly less so; the Champion, midway; the Early Black, somewhat resistant; and the McFarlin the most resistant. These values and terms are in such complete agreement with the results of several years of field observation as to indicate a direct correlation between the degree of attractiveness of a variety to the insect vector and the rapidity and extent of the spread of false blossom in that variety. One can hardly escape the converse conclusion that the apparent resistance of certain varieties is due, at least in part, to their unattractiveness to the blunt-nosed leaf hopper. It is possible that the McFarlin or other varieties finally will be found to possess, in addition, some degree of actual resistance to the false-blossom virus.

The task of securing a wider selection of cranberries which are resistant to the false-blossom disease involves the testing of many varieties. The method used in these experiments gives a quick and reliable test of one type of resistance which appears to be of practical importance.

SUMMARY

On the basis of field observations extending over several years, the five varieties of cranberries used in the experiments have been arranged in the following descending order of susceptibility to the false-blossom disease: Howes and Centennial, Champion, Early Black, and McFarlin.

Observation of the insects in cages where they had free choice of food plants placed these varieties in the same order in regard to their attractiveness to the carrier of false blossom.

The attractiveness of cranberry varieties to the disease vector appears to be correlated with the rate of spread of the disease on these varieties under field conditions and, conversely, the apparent resistance of certain varieties to the disease appears to consist, at least in part, of resistance to the vector.

A convenient method is described for testing this type of varietal resistance.

⁸ Experiments by Wilcox (8) indicate that changes occur in August which make even a late-maturing variety more resistant to false-blossom infection.

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FURTHER STUDIES OF SIZE AND SHAPE OF PLOT IN RELATION TO FIELD EXPERIMENTS WITH SUGAR BEETS¹

By F. R. IMMER, *associate geneticist*, and S. M. RALEIGH, *agent*, *Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The literature relating to standardization of field experiments with sugar beets (*Beta vulgaris* L.) was reviewed briefly by the senior writer in a previous paper.² While the literature dealing with the question of the most efficient size and shape of plots for testing varieties of some crops is very extensive, little has been published on the proper size of plots to be used in sugar-beet agronomy. So far as the writers are aware, the work reported in the paper just cited was the first extensive study of the relative efficiency of plots of varying size and shape in agronomic trials with sugar beets. The present paper presents further data on the same problem, as well as on the relation of stand to yields and the use of the regression of yield on stand as a means of estimating the error of the experiment.

MATERIALS AND METHODS

The experimental design of this study was essentially the same as that previously employed.^{3 4} A field of sugar beets, 110 by 330 feet, planted to the Kleinwanzleben Normal brand or variety in 1931, was chosen for the experiment. The field had been cropped in a uniform manner in previous years and cultural practices were the same over the entire area in 1931. On May 13 the beets were planted in rows 22 inches apart. At thinning time the beets were mechanically cross-blocked and then thinned to a single beet per 12 inches of row. At harvest time the field was marked out into plots 2 rods (33 feet) long. The field then contained 600 single-row plots 2 rods long and was 60 rows wide by 330 feet long, each row being subdivided into 33-foot units. All beets adjacent to skips in the rows were removed at harvest time and weighed separately for each plot. The remaining beets, namely, those with normal competition on all sides, were then harvested, counted, cleaned, and weighed. The average weight per root of these normally competitive beets in each unit plot was determined and then multiplied by 33 to obtain the theoretical yield of the plot on the basis of a perfect stand. The stand prior to removal of plants adjacent to skips was 83.3 percent perfect. After removal of all beets adjacent to skips the stand of normally competitive beets was 62.8 percent.

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² IMMER, F. R. A STUDY OF SAMPLING TECHNIC WITH SUGAR BEETS. Jour. Agr. Research 44: 633-647. 1932.

³ IMMER, F. R. (See footnote 2.)

⁴ ——— SIZE AND SHAPE OF PLOT IN RELATION TO FIELD EXPERIMENTS WITH SUGAR BEETS. Jour. Agr. Research 44: 649-668, illus. 1932.

The analysis of variance ⁵ method was used in analyzing the data. The statistical technic employed has been discussed thoroughly in previous papers.^{6 7}

EXPERIMENTAL RESULTS

ANALYSIS OF YIELD DATA

The analysis of variance of yield was made on the assumption of five varieties or treatments to be tested. The experimental design is one of randomized blocks. The different sizes and shapes of plots studied were the same as were used before.⁷ In table 1 is given the analysis of variance for weight of roots per plot of one row 2 rods long, expressed on the perfect-stand basis.

TABLE 1.—Analysis of variance of weight of beets in single-row plots 2 rods long

Variation	Degrees of freedom	Sum of squares	Mean square ^a	Standard deviation	z^b
Between blocks.....	119	7,803 8239	65 5784	8.0980	} 0.3004
Within blocks.....	480	15,310 1400	31.8961	5.6477	
Total.....	599	23,113 9639			

^a Mean square or variance (S.D.)² = $\frac{\text{Sum of squares}}{\text{Degrees of freedom}}$

^b z = one half the difference between the natural logarithms of the 2 variances

Since there were 600 unit plots in the test there would be 599 degrees of freedom for the total. The 120 blocks of 5 hypothetical varieties each would contribute 119 degrees of freedom, leaving 480 degrees of freedom for variation within blocks. The value of z , used in testing the significance of the difference between variances between and within blocks exceeded the 1 percent point, and it is concluded that variation in weight between blocks was greater than that within blocks. A significant reduction in the error (variation within blocks) has resulted, therefore, by removing the block effect. The mean square for variation of a single plot within blocks (31.8961) was greater than the mean square calculated in the previous study ⁸ in 1930 (21.9744). The average stand of normally competitive beets was 85 percent in 1930 and 63 percent in 1931. The variance (mean square) within blocks increased very nearly proportionately to the inverse ratio of the stand. The mean square between plots within blocks, as pointed out previously,⁸ is made up of two components, (1) variation due to inherent soil differences between plots and (2) the number of beets per plot actually harvested.

In a similar way the variance of 3-row plots 2 rods long may be calculated when only the central row of each plot was considered harvested. By so doing it would be possible in an actual experiment to correct for differential competition between adjacent varieties by removing the border rows. The analysis of variance is given in table 2.

⁵ FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 p., illus. Edinburgh and London, 1930

⁶ IMMER, F. R. (See footnote 2)

⁷ ——— (See footnote 4.)

⁸ IMMER, F. R. (See footnote 2.)

TABLE 2.—*Analysis of variance of weight of beets in 3-row plots 2 rods long of which only the central row was harvested*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	<i>z</i>
Between blocks.....	39	1,601.8942	41.0742	6.4089	} 0.0699
Within blocks.....	160	5,713.7480	35.7109	5.9759	
Total.....	199	7,315.6422			

There are now only 200 plots (of 3 rows each), which will give 199 total degrees of freedom. Since each block of 5 hypothetical varieties will require 15 single rows, there will be 40 such blocks, giving 39 degrees of freedom and leaving 160 for variation within blocks.

The variance within blocks (35.7109) is again higher than in the previous study⁹ made in 1930 (23.5453), due partly to the reduced stand in 1931. The value of *z* indicates that the variation between blocks was not significantly greater than the variation within blocks, since the observed value of *z* (0.0699) did not exceed the 5 percent point.

Considering hypothetical plots 1, 2, 3, 4, 6, and 12 rows wide and 2, 4, 10, and 20 rods long, similar analyses of variance were made and the variances between the 5 plots within blocks determined. For plots 3 or more rows wide a single border row could be discarded on either side of the plot and the analysis of variance made on this basis. That differential competition does exist between adjacent rows of two different varieties of beets under some conditions and must be eliminated has been demonstrated.¹⁰ In table 3 are given the standard deviations in percentage of the mean for these combinations of width and length of plot for 1931 and the average of 1930 and 1931. The average standard deviations were obtained by averaging the variances (mean squares) and extracting the square root.

TABLE 3.—*Standard deviation, in percentage of the mean, of yields of plots varying in size and shape in 1930 and 1931*

ENTIRE PLOT HARVESTED												
Standard deviation of yields (percent) for plots of indicated width (rows)												
Length of plot (rods)	1931						Average of 1930 and 1931					
	1	2	3	4	6	12	1	2	3	4	6	12
2.....	9.52	7.21	5.90	5.56	5.53	4.56	9.34	7.06	5.95	5.92	5.94	4.81
4.....	7.08	5.49	4.03	4.10	4.44	3.34	7.25	5.64	4.76	4.83	5.14	4.41
6.....	5.36	4.36	3.27	3.26	3.81	2.73	5.58	4.54	3.89	3.66	4.58	3.34
10.....	4.23	3.40	2.56	2.51	3.38	2.49	4.57	3.79	3.12	3.68	4.21	2.89
CENTRAL ROWS HARVESTED												
2.....			10.17	7.21	6.39	4.79			9.85	7.70	6.88	4.90
4.....			7.35	5.28	5.37	3.55			7.76	5.38	5.95	4.15
6.....			6.02	4.28	4.65	2.84			6.11	4.50	5.46	3.51
10.....			4.87	3.07	4.12	2.59			5.30	3.75	5.01	3.32

⁹ IMMER, F. R. (See footnote 4.)¹⁰ Unpublished data

As was to be expected, the standard deviation in general decreased as the size of plot was increased. When the entire plot was harvested in 1931 the error decreased more rapidly by increasing the length of the plot from 2 to 4 rods than by increasing the width. There seems to have been little difference between the effect of increasing the length from 10 rods to 20 rods and that of doubling the width. When the central rows alone were harvested, increasing the width of the plot reduced the standard error more rapidly than increasing the length of the plot.

When the entire plot was harvested, the average standard deviations for 1930 and 1931 showed very little difference between the efficiency of increasing the length of the plot and that of increasing the width as a means of reducing the standard deviation. When only the central rows were harvested, increasing the width of the plot was, in general, decidedly the more efficient method.

The efficiency of plots of varying size and shape was calculated by comparing the variances, per unit area, of plots differing in size and shape. A single-unit-plot basis for the study was secured by multiplying the variances per plot by the number of units (single rows 2 rods long) in each plot. Accepting the variance within blocks of single plots 2 rods long as a standard, the efficiency of plots of other sizes and shapes was determined by dividing the variances of a single-row plot 2 rods long by the variances of plots of other sizes and shapes, calculated on a unit basis. For example, in 1930 and 1931 the average variance within blocks, in percentage of the mean, for two-row plots 2 rods long, was 49.8268. The variance within blocks, for single-row plots 2 rods long, was 87.2699. To place the study on a unit basis, the variance of the 2-row plots was multiplied by 2, giving 99.6536. By dividing 87.2699 by 99.6536, 87.6 percent was obtained as the efficiency of the 2-row plots compared with the single-row plots, both being 2 rods long. In a similar manner the efficiencies of other combinations were determined. Table 4 shows the efficiency of these plots in 1931 and the average efficiency in 1930 and 1931.

TABLE 4.—Percentage efficiency in use of land of plots varying in size and shape in 1930 and 1931

ENTIRE PLOT HARVESTED												
Percentage efficiency of plot of indicated width (rows)												
Length of plot (rods)	1931						Average of 1930 and 1931					
	1	2	3	4	6	12	1	2	3	4	6	12
2..	100.0	87.2	86.8	73.4	49.5	36.4	100.0	87.6	82.2	62.2	41.2	31.4
4..	90.4	75.1	92.9	67.5	38.3	33.8	83.0	68.5	64.3	46.8	27.6	31.2
10..	63.1	47.7	56.7	42.6	20.8	20.2	56.1	42.3	38.5	32.6	13.9	13.0
20..	50.7	39.2	46.0	35.8	13.2	12.2	41.8	30.4	29.8	16.1	8.2	8.7
CENTRAL ROWS HARVESTED												
2..			29.2	43.5	37.0	32.9			30.0	36.8	30.7	29.2
4..			28.0	40.6	26.2	30.0			24.2	37.6	20.5	21.1
10..			16.7	24.8	14.0	18.7			15.6	21.5	9.8	11.8
20..			12.7	24.1	8.9	11.3			10.4	15.5	5.8	6.6

When the entire plot was harvested the efficiency decreased as the size of plot was increased. The standard error decreased as the plot size was increased (table 3). This decrease was not proportional to the increased area of the plots, however, but resulted in a lower efficiency for the larger plots. It would be more advantageous, therefore, to use small plots and replicate sufficiently to reduce the error to the level desired, than to use larger plots and fewer replications. By so doing the total area of land required for the test would be kept at the minimum.

When only the central rows were harvested, the 4-row plots were decidedly the most efficient. When border rows are removed, more land is required for 3-, 6-, or 12-row plots than for 4-row plots in order to reduce the standard errors to the same level; therefore there would be little or no advantage in using the 3-row in preference to the 4-row width of plot. It is likewise questionable whether the slight decrease in the cost of planting and harvesting a smaller number of 6- and 12-row plots as compared with a larger number of 4-row plots would compensate for the increased cost of caring for the greater area of

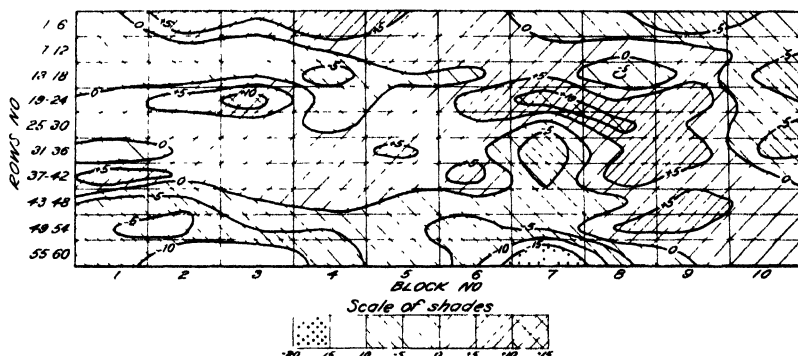


FIGURE 1.—Contour map of weight of roots from one hundred 6-row plots each 2 rods long, contour lines drawn through the points deviating by -15 , -10 , -5 , 0 , $+5$, and $+10$ percent from the mean weight

land needed for the wider plots. When border rows are removed the 4-row plot seems to be the ideal width. Apparently it makes little difference whether these are 2 or 4 rods long. The latter length might be preferable, especially since poor stands are sometimes encountered.

In figure 1 is shown a contour map of soil heterogeneity on the field from which the following data were obtained. The average yield per row was determined for a field of one hundred 6-row plots each 2 rods long. Assuming this average yield to be found at the middle of each plot, the points where the yields were average, where they were 5 and 10 percent above the mean, and where they were 5, 10, and 15 percent below the mean were obtained by linear interpolation. Points where the yields showed similar deviations were joined by continuous lines, and the contour map shown in figure 1 was thus constructed.

The systematic nature of soil heterogeneity, as it influenced yield, is apparent. Toward the left side of the diagram the contour lines tended to parallel the rows to a very pronounced degree. Had the direction of these contours been known prior to planting time it would have been more advantageous to plant the seed in rows at right

angles to the direction of the yield contours. Toward the right side of the diagram the contours no longer paralleled the direction of planting to the same degree and it would have made little difference in which direction the rows were planted. The soil on this field appeared very uniform in type. It would have been impossible to predict the location or direction of these contour lines prior to the analysis of the yield data. In spite of the apparent uniformity of the soil type, differences as great as 20 percent can be found in the inherent yielding capacity of the soil in areas only 65 feet apart. The need of replication under such conditions is apparent. Considering the systematic nature of the soil heterogeneity the necessity of randomizing the arrangement of plots in a field trial is obvious also.

REGRESSION OF YIELD ON STAND

Knowing the total number of beets in a plot and the weight of these beets, it is possible to calculate the expected weight from the number of roots. It was assumed in this study that five varieties were to be tested in 4-row plots each. The regression of yield on stand was calculated within such blocks of 20 rows, each 2 rods long. The regression equation, expressing the expected weight of all beets in a single plot 2 rods long, within blocks of 20 rows each, the stand being known, was found to be

$$W' = 50.7022 + 1.566210 (r' - \bar{r}'),$$

where W' is the expected weight of all beets and r' is the total number of beets in a single-row plot. The regression coefficient within blocks was calculated from the variance for root number and covariance for number of roots multiplied by weight within blocks, as discussed in a previous paper^{11 12}. The covariance within blocks is calculated in the same manner as variances, that is, by subtracting the sum of products due to blocks from the total sum of products, leaving the sum of products within blocks. In table 5 the significance of the regression coefficient (1.566210) is tested.

In testing the significance of the linear regression the observed value of z greatly exceeded the 1-percent point, and it is concluded that the regression undoubtedly was significant. The observed value of z found in testing the significance of the quadratic regression also exceeded the 1-percent point, and it is concluded that the quadratic regression

$$W' = 50.7022 + 2.503668 (r' - \bar{r}') - 0.017923 (r'^2 - \bar{r}'^2)$$

was significant also. Variation due to quadratic regression minus linear regression may be compared with variation due to deviations from quadratic regression in order to determine whether the quadratic relationship supplied more information than did the linear.

The value of z for this comparison (0.5962) did not reach the 5-percent point, and it may be concluded that the quadratic regression was not significantly better than the linear. The stands varied from about 50 percent (only 2 of the 600 plots had less than a 50-percent

¹¹ IMMERS, F. R. (See footnote 2.)

¹² ——— (See footnote 4.)

stand) to 100 percent. Within this range the regression of total weight of all beets on the total number of roots was essentially linear.

TABLE 5.—*Tests of significance of linear and quadratic regression of weight of all beets on total number of beets and of increased accuracy of quadratic over linear regression*

LINEAR REGRESSION				
Variation due to—	Degrees of freedom	Sum of squares	Mean square	<i>z</i>
Linear regression	1	14, 215 5929	14, 215 5929	} 3 2945
Deviations from linear regression	569	11, 126 4411	19 5544	
Total within blocks	570	25, 342 0340	44 4507	

QUADRATIC REGRESSION				
Quadratic regression	2	14, 279 7662	7, 139, 8831	} 2.9521
Deviations from quadratic regression	568	11, 062 2678	19 4758	
Total within blocks	570	25, 342 0340	44 4507	

QUADRATIC AND LINEAR REGRESSION COMPARED				
Quadratic minus linear regression	1	64 1733	64 1733	} 5962
Deviations from quadratic regression	568	11, 062 2678	19 4758	
Deviations from linear regression	569	11, 126 4411	19 5544	

In agronomic trials beets adjacent to skips in the row are usually removed before harvest, and only plants with normal competition on all sides are harvested. The yield is then expressed on the basis of a perfect stand on the assumption that the average weight per plant harvested is the weight which would have been secured if a perfect stand had been obtained. The regression of the weight of competitive beets on the number of normally competitive beets (within blocks 20 rows wide and 2 rods long) was found to be given by

$$W = 37.2497 + 1.736746 (r - r_0),$$

where W is the predicted weight of the competitive beets and r is the number of normally competitive beets per single-row plot. In table 6 is given the test of significance of the regression coefficient.

TABLE 6.—*Test of significance of linear regression, within blocks, of weight of normally competitive beets on number of normally competitive beets in single-row plots 2 rods long*

Variation due to—	Degrees of freedom	Sum of squares	Mean square	<i>z</i>
Linear regression	1	34, 935.0753	34, 935 0753	} 3.9548
Deviations from regression	569	7, 297.6757	12 8254	
Total within blocks	570	42, 232.7510	74 0925	

The observed value of z exceeded the 1-percent point, and it is concluded that the regression coefficient was significant. The mean square due to deviations from regression within blocks may be used as an estimate of the variance due to error, where "error" means deviation of the observed yields from the expected yields estimated from the regression relationship.

Another possible comparison presents itself, that obtained by multiplying the yield of all beets per plot, irrespective of skips, by a factor to convert the yield of each plot to a perfect-stand basis, as is done for competitive beets. After so raising the yield per plot, irrespective of skips, to a perfect-stand basis the error may be calculated in the usual manner. Since the regression of yield on stand was linear, such a practice would be as correct as raising the yields of competitive beets to a perfect-stand basis. The variance within blocks was found to be 31.5636. This is slightly lower than the variance calculated for perfect stands of competitive beets, but not significantly so. The slight increase in precision may be accounted for by the fact that the number of beets available was greater when all beets were taken than when only competitive beets were harvested. The slight deviation from linear regression was not great enough to offset the greater number of beets available when no correction was made for skips.

SUMMARY

Further studies are reported on size and shape of plot in relation to field experiments with sugar beets.

Standard errors, in percentage of the mean, decreased as the size of plots was increased.

When the entire plot was harvested, efficiency in the use of the land decreased as the size of plots was increased. When a single border row was removed on each side of the plot, the 4-row plot was decidedly the most efficient.

The regression of yield of all plants in a plot, irrespective of skips, on the total number of beets was essentially linear.

EFFECT OF EXCESS CARBON DIOXIDE ON GROWING MUSHROOMS¹

By EDMUND B. LAMBERT

Associate pathologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In mushroom culture the problem of ventilation has always been considered important, but so far as the writer is aware there has been no experimental work done on it. It is a common observation among growers employing underground caves that mushrooms have a tendency to "sulk" in certain pockets in the caves. It has also been known for many years that they have a tendency to grow abnormally long stems and small caps when confined in a limited atmosphere under a glass jar or similar container.

A consideration of these observations led the writer to make a series of experiments in which cultivated mushrooms (*Agaricus campestris* L.) of the white and cream varieties were grown in a modified atmosphere. Systematic observations were also made on the carbon dioxide content of commercial mushroom houses and caves under different conditions in order to determine the practical significance of the results of these experiments.

METHODS

The atmosphere was modified by slowly running carbon dioxide, nitrogen, and oxygen in different amounts and combinations into glass jars inverted over clumps of mushrooms growing on a standard mushroom bed. In some cases the gases were introduced into the compost beneath the clumps of mushrooms rather than into containers above the bed. The gases were obtained in standard commercial cylinders, and the flow was regulated by means of reducing valves, pinchcocks, and bubbling bottles. The arrangement of the apparatus is shown in figure 1. The only gases measured were carbon dioxide and oxygen. With the Orsat gas analysis apparatus used in these experiments the smallest portion of gas that it was feasible to determine was approximately 0.2 percent. Judging from the determination of replicate samples, the error inherent in the use of this apparatus is not more than 0.1 percent.

The effect of different treatments on the growth of the mushrooms was determined by weighing and making outline drawings of the four largest mushrooms in treated clumps and comparing these with similar data from untreated clumps having apparently an equal chance at the start.

It was necessary to exercise considerable care in selecting comparable clumps of mushrooms. Most of the experiments were made at temperatures approximating 15° C.

¹ Received for publication July 3, 1933, issued October 1933.

Except where otherwise specified, the atmosphere surrounding the clumps of untreated mushrooms, which were used as checks, contained less than 0.2 percent carbon dioxide and approximately 20.8 percent oxygen. In the interstices of a normal section of the compost under an actively growing clump of mushrooms there was approximately 20 percent oxygen, and the carbon dioxide content ranged from 0.3 to 0.4 percent.

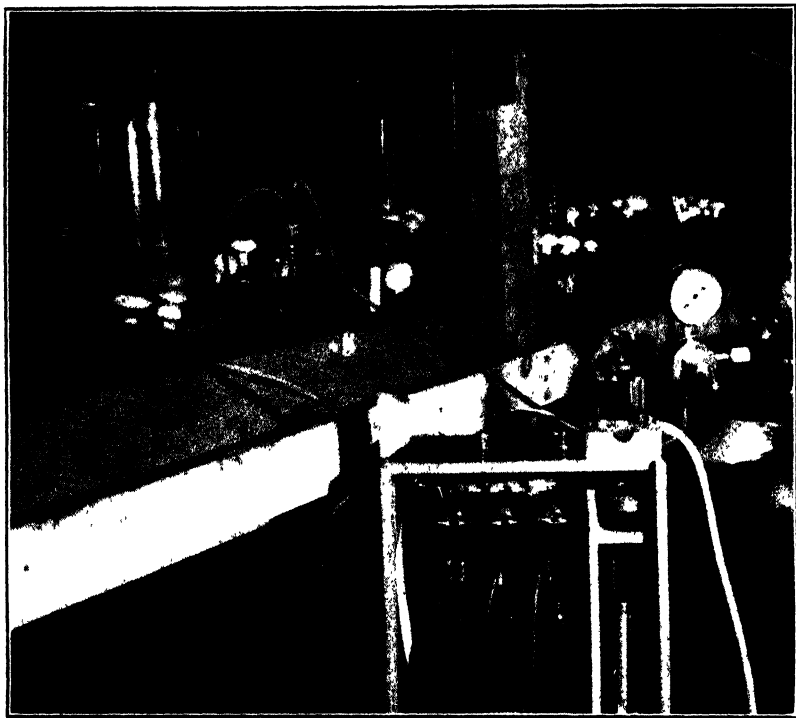


FIGURE 1.—Apparatus used for altering and testing the carbon dioxide and oxygen content of air surrounding growing mushrooms.

RESULTS OF EXPERIMENTS

EXPERIMENTAL SERIES NO. 1

The first group of experiments indicates that mushroom growth is arrested by diluting the atmosphere surrounding the caps and the mycelium with carbon dioxide, but there is no evidence to indicate whether the stunting is due to high carbon dioxide concentration or to the dilution of oxygen caused by the addition of carbon dioxide, nor is it clear whether the stunting is due primarily to the modification of the atmosphere surrounding the sporophores above ground or the mycelium in the compost.

EXPERIMENT 1-A.—Carbon dioxide was run slowly into a jar over a clump of button mushrooms for 5 days. The concentration of carbon dioxide varied from 6 to 18 percent, and that of oxygen from 16 to 19 percent. In the atmosphere of the compost beneath this jar the carbon dioxide content was 4.6 percent and the oxygen content 20.4

percent. As a result of this treatment the growth of the button mushrooms was completely arrested, while similar clumps outside of the jar

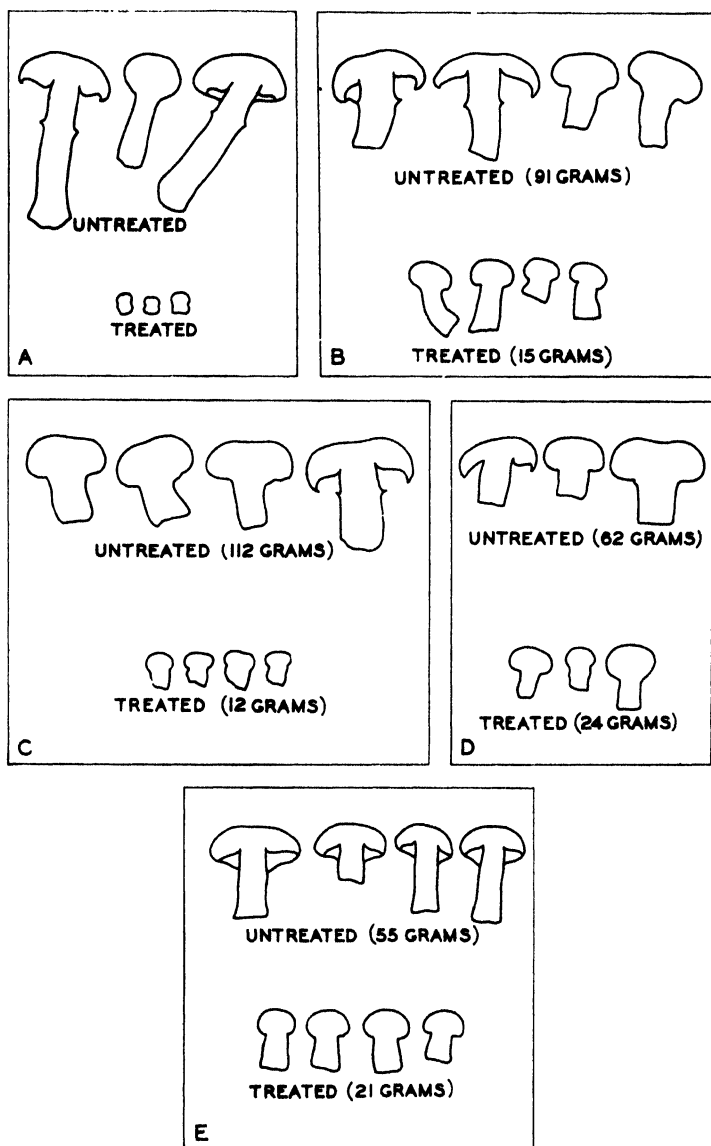


FIGURE 2.—Effect on the growth of mushroom sporophores of diluting the atmosphere with high concentrations of carbon dioxide. Treated mushrooms in group A were subject to 8 to 18 percent carbon dioxide for 5 days; group B, carbon dioxide 7.2 to 9.4 percent; C, carbon dioxide 8 to 9.6 percent; D, carbon dioxide 10.8 to 20 percent; E, carbon dioxide 15.6 to 22.4 percent. Untreated mushrooms in all had a comparatively equal start but were only subject to normal carbon dioxide in house, less than 0.1 percent.

grew to maturity. Figure 2, A, shows the comparative sizes of treated and untreated mushrooms at the end of 5 days.

EXPERIMENT 1-B.—Carbon dioxide was run into a jar over button mushrooms more slowly than in experiment 1-A. The concentrations of carbon dioxide in the atmosphere under the jar ranged from 7.2 to 9.4 percent; the range of the oxygen concentration was 19.0 to 20.2 percent. Growth was arrested, as shown in figure 2, *B*.

EXPERIMENT 1-C.—Carbon dioxide was run into a jar over button mushrooms, as in experiment 1-B. The carbon dioxide concentration of the atmosphere ranged from 8.0 to 9.6 percent, and the oxygen value ranged from 19.0 to 20.4 percent. The carbon dioxide content of the atmosphere in the compost beneath the clump of mushrooms ranged from 3.8 to 4.0 percent, while the oxygen remained at 20.2 percent. The results of this treatment are shown in figure 2, *C*.

EXPERIMENT 1-D.—A comparatively rapid flow of carbon dioxide was run into a jar over a clump of mushrooms. The carbon dioxide concentration in the jar ranged from 10.8 to 20.0 percent, while the oxygen was so diluted that it ranged from 16.8 to 18.4 percent. The atmosphere of the compost beneath the jar contained 6.0 to 6.6 percent carbon dioxide and 19.0 to 19.6 percent oxygen. The effect of this treatment on the growth of the mushrooms under the jar is shown in figure 2, *D*.

EXPERIMENT 1-E. A comparatively rapid flow of carbon dioxide was passed into the jar. The carbon dioxide content of the atmosphere in the jar was 15.6 to 22.4 percent, the weighted average being 18.4 percent. The oxygen content ranged from 15.0 to 17.6 percent and the weighted average was 16.6 percent. The effect of this treatment on the growth of the mushrooms is shown in figure 2, *E*.

EXPERIMENTAL SERIES NO. 2

The second group of experiments shows that moderate dilution of the atmosphere with nitrogen has little effect on the growth of the mushrooms, whereas dilution with carbon dioxide even in the presence of an excess of oxygen causes a distinct stunting of the mushrooms. It would seem from these results that the increase in the concentration of carbon dioxide rather than the dilution of oxygen is the important factor.

EXPERIMENT 2-A.—Nitrogen was run into the jar to dilute the oxygen. The oxygen content ranged from 7.8 to 20.0 percent, and the carbon dioxide concentration ranged from 0.3 to 0.7 percent. The average oxygen concentration for the entire experiment was 15.7 percent. The nitrogen content was not determined. The growth under these conditions compared favorably with that under jars arranged as checks with similar concentrations of carbon dioxide, 0.4 to 0.8 percent, but with very slight dilutions of oxygen, 20.1 percent. This is shown in figure 3, *A*.

EXPERIMENT 2-B.—This experiment was essentially a duplicate of experiment 2-A. The concentration of carbon dioxide ranged from 0.2 to 1.0 percent, while the oxygen content ranged from 10.4 to 19.6 percent, averaging 16.3 percent. The effect of this treatment on growth is shown in figure 3, *B*.

EXPERIMENT 2-C.—In this experiment both carbon dioxide and oxygen were run into the jar in order to obtain a high concentration

of carbon dioxide in the presence of an excess of oxygen. The oxygen concentration ranged from 25.8 to 26.4 percent, while the carbon dioxide concentration ranged from 7.6 to 9.2 percent. The effect of the treatment is illustrated in figure 3, *C*.

EXPERIMENT 2-D.—This experiment was performed in an attempt to duplicate experiment 2-C. Carbon dioxide concentration ranged from 16.0 to 22.6 percent and oxygen from 26.4 to 26.6 percent. The effect on growth and weight are shown in figure 3, *D*.

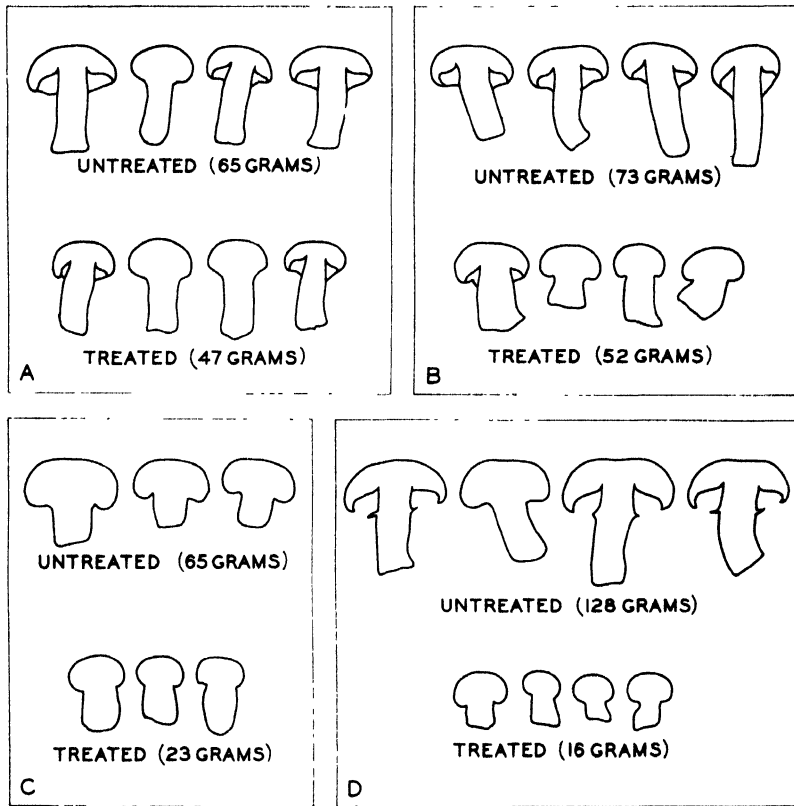


FIGURE 3.—Group *A* and group *B* show the effect of diluting the atmosphere with nitrogen. Groups *C* and *D* show the effect of high concentrations of carbon dioxide together with high concentrations of oxygen. Treated mushrooms in group *A* grew in an atmosphere with oxygen concentrations 7.8 to 20.0 percent and carbon dioxide 0.3 to 0.7 percent; group *B*, oxygen 10.4 to 19.6 percent, and carbon dioxide 0.2 to 1.0 percent; group *C*, oxygen 25.8 to 26.4 percent, carbon dioxide 7.6 to 9.2 percent; group *D*, oxygen 26.4 to 26.6 percent, carbon dioxide 16.0 to 22.6 percent. Untreated mushrooms grew in normal carbon dioxide and oxygen content of house.

EXPERIMENTAL SERIES NO. 3

The third series of experiments indicates that the stunting effect of an excess of carbon dioxide is due principally to its effect on the caps rather than on the mycelium in the compost.

EXPERIMENT 3-A.—Carbon dioxide was run slowly into the compost under a clump of button mushrooms growing in the open air on the bed. The carbon dioxide content reached 1.0 percent, but there was

no appreciable effect on the growth of the mushrooms, as shown in figure 4, A.

EXPERIMENT 3-B.—Carbon dioxide was run into the bed more rapidly than in experiment 3-A, and a higher carbon dioxide content resulted, 6.4 to 9.0 percent; yet there was no appreciable effect on the growth of the mushrooms. (See fig. 4, B.)

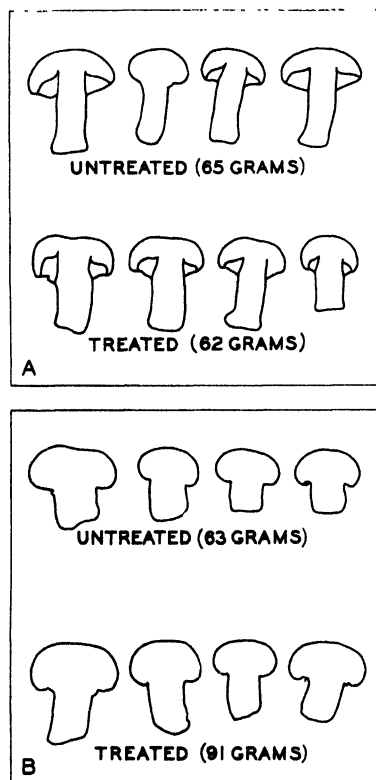


FIGURE 4.—Effect of high concentrations of carbon dioxide in the interstices of the compost under growing mushrooms. Under treated mushrooms in group A, carbon dioxide 1.0 percent; under treated group B, carbon dioxide 6.4 to 9.0 percent; untreated in both groups, carbon dioxide 0.3 to 0.4 percent. In all instances the sporophores grew in normal air

EXPERIMENT 4

In an attempt to revive the mushrooms stunted by high concentrations of carbon dioxide in experiments 1-B and 1-C, several stunted mushrooms were left on the bed after the jars were removed. Growth was not resumed, and it was evident that the young mushrooms had been killed by the exposure to excess carbon dioxide.

EXPERIMENTAL SERIES NO. 5

It would seem from the fifth series that there is a slight tendency for the stems of mushrooms to elongate at a concentration of carbon dioxide of about 1 percent. At 1.8 percent this tendency is quite marked.

EXPERIMENT 5-A.—The concentration of carbon dioxide ranged from 0.4 to 0.8 percent and averaged 0.5 percent. The effect on the growth of mushrooms is shown in figure 5, A.

EXPERIMENT 5-B.—The carbon dioxide concentration ranged from 0.2 to 1.0 percent and averaged 0.6 percent. The results are shown in figure 5, B.

EXPERIMENT 5-C.—The carbon dioxide concentration ranged from 2.2 to 2.6 percent and averaged 2.3 percent. The results are shown in figure 5, C.

EXPERIMENT 5-D.—The carbon dioxide concentration was 0.9 percent. Results are shown in figure 5, D.

EXPERIMENT 5-E.—The carbon dioxide concentration was 0.6 percent. The results are shown in figure 5, E.

EXPERIMENT 5-F.—(Replication of 5-E.) The carbon dioxide concentration was 0.6 percent; a comparison of the treated and untreated mushrooms is shown in figure 5, F.

EXPERIMENT 5-G.—This experiment was similar to the others in this series, except that it was made on a floor bed in a cave at St. Paul,

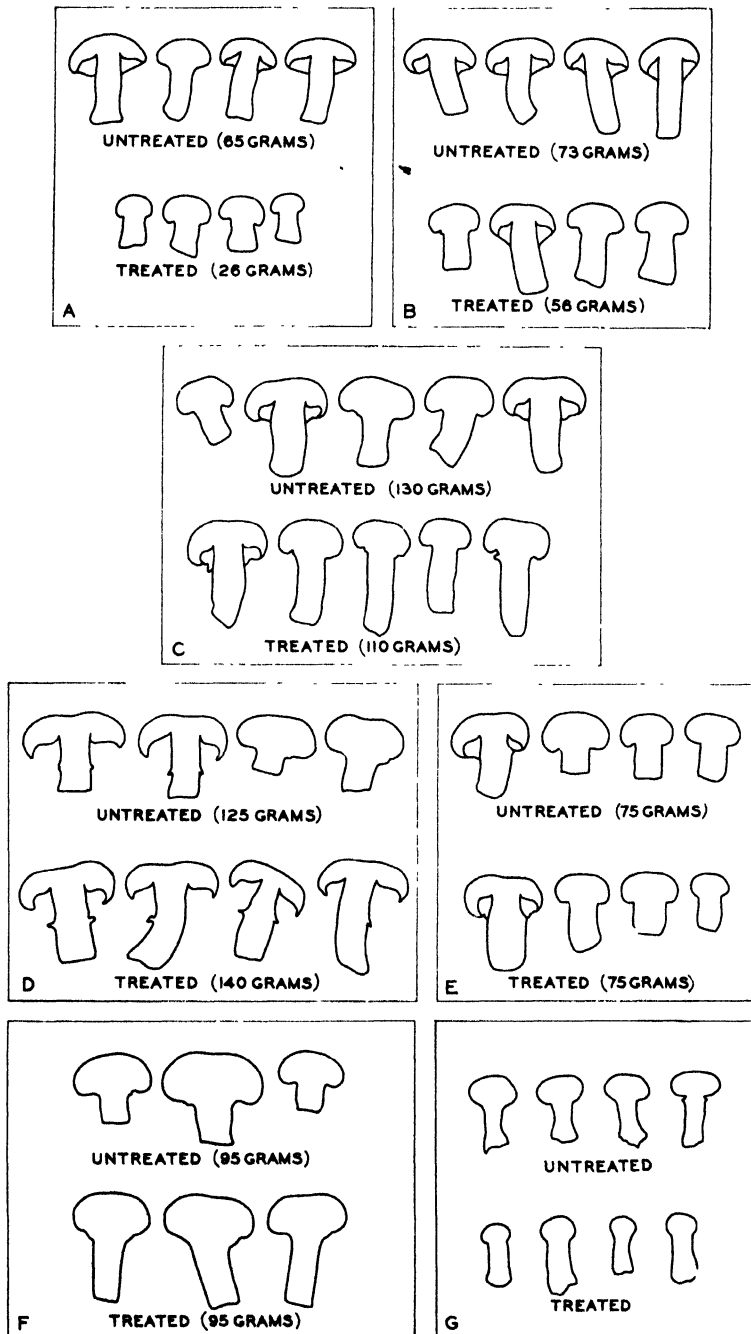


FIGURE 5.—Effect on the growth of mushrooms of small increases in concentration of carbon dioxide in the air. Treated sporophores in group *A* were subject to carbon dioxide average 0.5 percent; group *B*, carbon dioxide 0.6 percent; group *C*, carbon dioxide 2.3 percent; group *D*, carbon dioxide 0.9 percent; group *E*, carbon dioxide 0.6 percent; group *F*, carbon dioxide 0.6 percent; group *G*, carbon dioxide 1.8 percent untreated in all cases exposed to less than 0.1 percent carbon dioxide.

Minn., in 1931. The carbon dioxide accumulation under the jar reached 1.8 percent on the second day. The results are shown in figure 5, *G*.

EXPERIMENTAL SERIES NO. 6

In the sixth series a large excess of oxygen was run into jars containing clumps of mushrooms. There seemed to be a slight tendency for the mushrooms to be firmer and heavier in atmospheres containing a large excess of oxygen.

EXPERIMENT 6-A.—The oxygen content under the jar ranged from 36.7 to 38.4 percent; the carbon dioxide ranged from 0.7 to 0.8 percent. A comparison of treated and untreated mushrooms is shown in figure 6, *A*.

EXPERIMENT 6-B.—The oxygen content under the jar varied from 58 to 61 percent; the carbon dioxide content was 0.4 percent. A comparison of the treated with the check mushrooms is shown in figure 6, *B*.

EXPERIMENT 6-C.—The oxygen content was 28 percent and the carbon dioxide 0.6 per cent. A comparison of treated with check mushrooms is shown in figure 6, *C*.

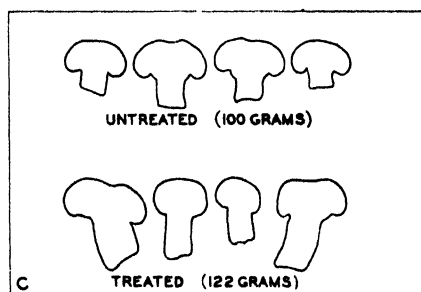
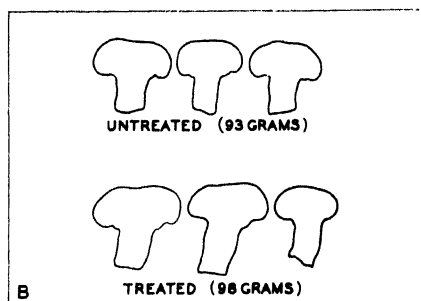
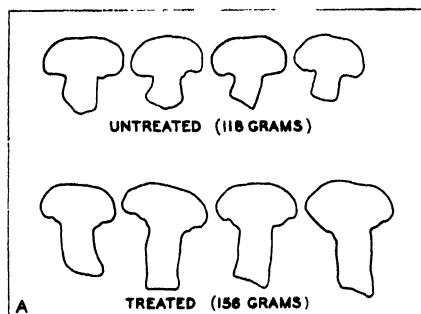


FIGURE 6—Effect of an excess of oxygen on the growth of sporophores. In group *A* the treated mushrooms grew in an atmosphere containing approximately 37 percent oxygen and about 0.8 percent carbon dioxide; group *B*, oxygen 58 to 61 percent, carbon dioxide 0.4 percent; group *C*, oxygen 28 percent, carbon dioxide 0.6 percent.

EXPERIMENT 7

An excess of oxygen was run into the compost under several clumps of mushrooms growing in the open air. There was no apparent stimulation from this treatment.

EXPERIMENT 8

Clumps of mushrooms were removed from the beds, and jars were inverted over these as well as over places in the bed containing tiny rudiments of mushrooms. After 5 days the carbon dioxide content under these jars averaged 0.2 percent. This is considerably

lower than the concentration in series 5, indicating, as would be expected, that the sporophores liberate carbon dioxide freely during active growth.

OBSERVATIONS IN CAVES AND COMMERCIAL HOUSES

In the summer of 1931, samples of air were analyzed from different parts of three sandstone caves devoted to mushrooms culture at St. Paul, Minn. In one cave the beds were spawned but not yet in bearing. In this cave the carbon-dioxide content averaged only about 0.2 percent, in spite of the fact that the cave had been closed up tightly for several days. In the second cave there were actively growing mushrooms, but the door was left open at night for ventilation. The air in this cave had approximately the same carbon dioxide content, 0.2 percent. The third cave was dug somewhat closer to the outside wall and could not be kept cool during hot weather unless the doors were tightly closed. There were growing mushrooms in this cave, and after the door had been closed for a few days the carbon dioxide would reach a concentration of 1 percent. The mushrooms in this cave had a slight tendency to grow long stems with small caps as compared with those in the second cave.

In May 1932, air was analyzed from the mushroom houses of three commercial growers in Chester County, Pa. In all cases mushrooms were growing on the beds. After the doors had been closed for several hours the carbon dioxide content of the air at the ground level varied from 0.2 to 0.5 percent, and at the top of the house from less than 0.2 to 0.4 percent. Opening the doors overnight reduced the concentration to less than 0.1 percent.

DISCUSSION AND CONCLUSIONS

The foregoing experiments should perhaps be considered as field rather than as laboratory experiments. With a plant as variable as the cultivated mushroom, and with the comparatively crude methods used for maintaining and determining the composition of the atmosphere, it was not possible to establish precisely the limits of carbon dioxide and oxygen concentration tolerated by the mushroom. In spite of these limitations, however, the results of most of the experiments were clear-cut and justify definite conclusions.

When compared to the germinating spores and growing mycelium studied by Lopriore,² Brown,³ Fellows,⁴ and Neal and Wester,⁵ the growing sporophores of cultivated mushrooms appear to be exceptionally sensitive to an excess of carbon dioxide in the air. Under the conditions of the experiments, concentrations of more than 5 percent carbon dioxide in the air arrested the growth of the sporophores and in some cases apparently injured them beyond recovery. At lower concentrations (2 percent, and perhaps even 1 percent) there was a tendency for the pileus to be retarded more than the stipe so that the mushrooms were out of proportion, having long stems and small caps.

It is of considerable interest that growing mushrooms are more sensitive to high concentrations of carbon dioxide surrounding the

² LOPRIORE, G. UEBER DIE EINWIRKUNG DER KOHLENSAURE AUF DAS PROTOPLASMA DER LEBENDEN PFLANZENZELLE. *Jahrb. Wiss. Bot.* 24, [531-636, illus. 1895.

³ BROWN, W. ON THE GERMINATION AND GROWTH OF FUNGI AT VARIOUS TEMPERATURES AND IN VARIOUS CONCENTRATIONS OF OXYGEN AND OF CARBON DIOXIDE. *Ann. Bot. [London]* 36: [257]-283, illus. 1922.

⁴ FELLOWS, H. THE INFLUENCE OF OXYGEN AND CARBON DIOXIDE ON THE GROWTH OF *OPHIOGLOUS GRAMINIS* IN PURE CULTURE. *Jour. Agr. Research* 37: 349-355, illus. 1924.

⁵ NEAL, D. C., and WESTER, R. E. EFFECTS OF ANAEROBIC CONDITIONS ON THE GROWTH OF THE COTTON-ROOT-ROT FUNGUS *PHYMATOTRICHUM OMNIVORUM*. *Phytopathology* 22: 917-920, illus. 1932.

sporophores above ground than to similar concentrations in the interstices of the compost occupied by the mycelium. A possible explanation for this is that the comparatively loosely woven structure of the sporophore allows a direct diffusion of gases into the air from most of the hyphae, whereas the mycelium in the compost is buried deeply in the organic material through which it is growing and is therefore only partly in contact with the atmosphere of the compost. It may also be of some significance that when the hyphae of the sporophores are growing and respiring actively the mycelium in the compost has already finished most of its growth and is chiefly active in absorbing and translocating nutrients. In any case there is no justification for assuming that the evidence presented applies to young actively growing mycelium, or to sporophores in storage.

Analyses of gas in commercial houses and caves indicate that in practice harmful concentrations of carbon dioxide occur when the caves or houses have been closed up tightly for 24 hours or more. Theoretically the excess of carbon dioxide may be removed by air currents, by diffusion, or by absorption. Harmful concentrations of carbon dioxide can be avoided during the winter by opening the doors or ventilators for a few hours each day. In the case of summer crops grown in water-refrigerated houses where the air is recirculated to minimize the introduction of warm outside air it would seem advisable to make the water in the cooling sprays alkaline in order to absorb the accumulating carbon dioxide, and also to open the ventilators at the top of the house to allow for the escape of carbon dioxide by diffusion.

The evidence that a moderate dilution of oxygen is not detrimental to the growth of *Agaricus* is in agreement with the work of others, notably that of Porodko⁶ and Brown,⁷ which indicates that aerobic microorganisms usually are more tolerant of moderate reductions in oxygen pressure than of excesses of carbon dioxide.

SUMMARY

By running carbon dioxide under bell jars inverted over growing mushrooms (*Agaricus campestris* L.) it was found that an accumulation of 5 percent or more of carbon dioxide in the air caused abnormal growth, stunting, and even death of the mushrooms. Further experiments indicate that this effect was due to the presence of carbon dioxide and not to the dilution of oxygen. It was also demonstrated that the harmful effect was due principally to the carbon dioxide surrounding the sporophores rather than to that in the interstices of the compost in the bed. Approximately 1 percent of carbon dioxide was the lowest concentration that was noticeably injurious. This concentration was not encountered in commercial mushroom houses or caves except where they had been closed up tightly for 24 hours or more. An abnormally high concentration of oxygen seemed to produce more compact and heavier mushrooms. It is evident that mushroom houses should be ventilated as much as possible without interfering with temperature and humidity control or causing excessive evaporation from the beds by cross drafts.

⁶ PORODKO, T. STUDIEN ÜBER DEN EINFLUSS DER SAUERSTOFFSPANNUNG AUF PFLANZLICHE MIKROORGANISMEN. Jahrb. Wiss. Bot. 41: 1-64. 1904.

⁷ BROWN, W. (See footnote 3)

EFFECT OF CERTAIN ENVIRONMENTAL FACTORS ON THE GERMINATION OF THE SPORANGIA OF *PHYSDERMA ZEA-MAYDIS*¹

By R. K. VOORHEES²

Assistant plant pathologist, Florida Agricultural Experiment Station

INTRODUCTION

In his studies of infection of corn plants by *Physotherma zae-maydis* Shaw, Eddins (2)³ noted that there was a wide variation in the incubation period following artificial inoculations, and concluded that these differences were probably due to the variation in time required for the sporangia to become exposed to conditions favorable for germination. He obtained higher percentages of infection in the field than in the greenhouse after artificial inoculation which indicated that certain environmental factors were more favorable for infection in the field.

According to Tisdale (6), the more essential factors influencing sporangial germination and zoospore formation of this organism are moisture, temperature, and fresh air. Additional factors that might possibly affect the development of a parasite are acidity of the medium, light, age of reproductive bodies, and gas content of the surrounding atmosphere. Several series of experiments were conducted in the laboratory and greenhouse to determine the effect of these factors on the germination of the sporangia in water cultures.

MATERIALS AND METHODS

Three hundred to five hundred sporangia collected from infected plants of corn (*Zea mays* L.) grown in 1932 were placed in each of several Syracuse dishes containing distilled water. These dishes in turn were placed in open chambers in the greenhouse, or on the laboratory table in moist chambers, in sealed desiccators, and in Novy improved anaerobic culture jars. The results given for each experiment represent an average of two trials run in duplicate. Unless otherwise stated, each experiment was conducted at a temperature between 27° and 30° C., which, according to Tisdale (6), comes within the range favorable for germination.

In a preliminary experiment the sporangia germinated equally well in tap water, distilled water, and corn-leaf extract. Therefore, in order to reduce to a minimum any variation in the acidity of the medium itself, distilled water was used in these experiments. Since it was also observed that the sporangia began germinating after 36 hours and showed the maximum percentage of germination at the end of 72 hours, the final counts were made in the remaining experiments

¹ Received for publication May 16, 1933; issued October 1933.

² Thanks are due Dr. A. H. Eddins for suggesting the investigation of this problem.

³ Reference is made by number (italic) to Literature Cited, p. 473.

after 72 hours. The percentage germination was obtained by counting a total of 100 apparently normal sporangia in each dish. The sporangia were considered to have germinated when the lid or cap of the resting spores opened in doorlike fashion or was carried at the apex of the protruding endosporangium, as described by Tisdale (6).

FACTORS INFLUENCING THE GERMINATION OF THE SPORANGIA

FRESH AIR

In this test the Syracuse dishes containing the sporangia were placed in open chambers, moist chambers, and sealed desiccators on a laboratory table. The moist chambers were not sealed, which permitted the air to pass in and out of the chambers in places on the rim where the tops did not fit down tightly. The desiccators were sealed tightly with Lubriseal to prevent the exchange of the air between the outside and inside of the containers. In the open chambers and moist chambers, 87 percent germination was obtained; in the sealed desiccators 86 percent germination was obtained. This indicates that it is not necessary for the sporangia to be exposed to a constant supply of fresh air.

LIGHT

Germination tests were made with sporangia of *Physoderma zeae-maydis* by placing them in Syracuse dishes exposed to different light conditions. Part of the dishes were left open in the laboratory and in the greenhouse, and others were enclosed in moist chambers. The temperatures at which the containers were kept in different intensities of light were not the same in every case, but all of them came within the range favorable for germination.

The results of two trials (table 1) show that the intensity of light greatly affects the percentage of germination. Of the sporangia which received light from a north window in the laboratory 87 percent germinated, but of those in a dark chamber without light none germinated. Sporangia germinated in the presence of light from an electric lamp. This was demonstrated by placing them in a dark refrigerator adjusted to 10° C. and equipped with a 50-watt Westinghouse daylight bulb. By means of a small fan the cold air was drawn into the chamber containing the sporangia and kept circulating over the electric lamp. Thus, a constant temperature of 33° was maintained in the chamber, and 75 percent germination was obtained. Sporangia placed in open and in closed moist chambers on top of a greenhouse did not germinate. The sporangia in the open chambers were exposed to direct sunlight, while those in the closed chambers received sunlight through the glass cover of the chambers. No germination was obtained when the sporangia were placed in open chambers in the greenhouse 1 foot from the top and on a bench 8 feet from the top. In this case the sporangia were exposed to sunlight entering through the glass top of the greenhouse. Recording thermographs installed in the immediate vicinity of the containers showed that the temperature was favorable for germination in each case. The mean temperatures are shown in table 1. With all other conditions apparently favorable for germination, the foregoing results seem to indicate that light plays an important part in the germination of the sporangia.

TABLE 1.—*The relation of light to the germination of the sporangia of Physoderma zeae-maydis*

Place of exposure	Temperature	Percentage germination
	°C	
Moist chamber on laboratory table	27	87
Darkened corner of laboratory	27	23
Complete darkness in chamber	30	0
Artificial light in dark chamber	33	75
Sunlight in closed moist chamber	32	0
Direct sunlight in open moist chamber	32	0
Greenhouse, 1 foot from top	30	0
Greenhouse, 8 feet from top	28	0

HYDROGEN-ION CONCENTRATION OF THE MEDIUM

The sporangia of *Physoderma zeae-maydis* were germinated in Syracuse dishes containing distilled water adjusted to different hydrogen-ion concentrations ranging from pH 2.1 to 8.1. The adjustments were made by adding the required number of drops of hydrochloric acid and sodium hydroxide of different normalities, the determinations being made with a Youden hydrogen-ion concentration apparatus. The sporangia germinated 88 percent at pH 7.4, which was the optimum reaction for germination. Germination was low at pH 3.1 and was inhibited at pH 2.5.

AGE OF THE SPORANGIA

Tisdale (6) has shown that the sporangia of *Physoderma zeae-maydis* remain viable over winter in the South. Eddins (2), in his studies on the longevity of the sporangia of this organism, has shown that sporangia stored for 3 years under different conditions were capable of causing from 41 to 85 percent infection when sprayed on corn plants.

In the present experiment, the sporangia were germinated in distilled water by the method described earlier in this paper. Some of the sporangia used were from the same bottles stored in the laboratory from which Eddins (2) had obtained the sporangia he used to inoculate corn plants. The sporangia stored in laboratory bottles for 1, 2, and 3 years germinated 70, 46, and 0 percent, respectively, as shown in table 2. The 3-year-old sporangia showed no germination in the laboratory but sporangia of the same age produced 41 percent infection when sprayed on corn plants in the field (2). This difference between the germination and the percentage of infection obtained may have been due to the stimulating effect of the presence of plant tissues on the spores, as noted by Durrell (1) for the spores of *Basisporium gallarum*, and Platz et al. (5) for the spores of *Ustilago zeae*.

TABLE 2.—*The effect of age of the sporangia of Physoderma zeae-maydis on their germination in distilled-water cultures*

Year sporangia were collected	Age of sporangia	Percentage of germination	Year sporangia were collected	Age of sporangia	Percentage of germination
1932	0 months	87	1929	3 years	0
1931	1 year	70	1928	4 years	0
1930	2 years	46			

GAS CONTENT OF THE SURROUNDING ATMOSPHERE

OXYGEN CONTENT

Platz (4) found that the percentage of germination of the spores of *Ustilago zaeae* was affected by various percentages of oxygen and that the spores would not germinate in the absence of oxygen.

In testing the effect of various concentrations of oxygen in the surrounding atmosphere on the percentage of germination of *Physoderma zaeae-maydis*, Syracuse dishes containing the sporangia were placed in Novy jars of a known volume, closed with ground-glass tops, and sealed with Lubrisal. The air was exhausted from the jars by means of a vacuum pump and the oxygen admitted into the jars from a gas cylinder was measured with gas burettes. After the desired percentage of oxygen had been admitted into each jar, air was admitted to make up the total volume. Thus, the gas in the jars was at normal atmospheric pressure. The pH reaction was determined at the beginning and at the end of the experiments. An attempt was made to germinate the sporangia of *Physoderma zaeae-maydis* in the absence of oxygen by removing it from the Novy jars by the pyrogallic acid method (7).

The results (table 3) show that the percentage of germination of the sporangia is affected by the percentage of oxygen contained in the atmosphere. The highest percentage of germination was obtained in an atmosphere containing 20 percent of oxygen. It is interesting to note that this was the same percentage of germination as that obtained in the checks in normal atmosphere, which contains approximately 20 percent of oxygen by volume. The sporangia did not germinate in the absence of oxygen, whereas they germinated 80 percent in a normal atmosphere.

TABLE 3.—The effect of oxygen on the germination of the sporangia of *Physoderma zaeae-maydis*, and its effect on the pH of the medium, which was 6.9 at the beginning of the experiment

Percentage of oxygen	Percentage of germination of sporangia	pH reaction of medium at end of experiment	Percentage of oxygen	Percentage of germination of sporangia	pH reaction of medium at end of experiment
Check.....	80	6.9	20.....	80	7.6
0.....	0	6.1	25.....	76	7.8
5.....	72	7.0	30.....	64	8.0
10.....	73	7.2	35.....	58	8.2
15.....	75	7.4			

The atmosphere containing higher percentages of oxygen produced greater alkalinity of the medium. In the oxygen-free atmosphere, the hydrogen-ion concentration of the medium was changed from pH 6.9 to 6.1. An atmosphere containing 20 percent of oxygen produced in distilled water a hydrogen-ion concentration of 7.6, at which the maximum germination was obtained. This germination is comparable to that obtained at 7.4 produced by a known normality of sodium hydroxide in normal atmosphere.

CARBON DIOXIDE CONTENT

Sporangia of *Physoderma zae-maydis* were exposed to atmospheres containing various percentages of carbon dioxide. This was done in the same manner as in the preceding experiment with oxygen. The results of the two trials (table 4) show that the percentage of germination of the sporangia is affected by the percentage of carbon dioxide. Atmospheres containing 10 percent of carbon dioxide produced the highest percentage of germination, which is approximately the same as that obtained in a normal atmosphere and in that containing 20 percent of oxygen. No germination was obtained in the presence of a barium hydroxide solution, which probably absorbed all of the carbon dioxide in the containers. This same experiment was performed by Durrell (1), who found that spores of *Basisporium gallarum* also failed to germinate in the absence of carbon dioxide.

TABLE 4. *The relation of carbon dioxide to the germination of the sporangia of Physoderma zae-maydis, and its effect on the pH of the medium, which was 6.6 at the beginning of the experiment*

Percentage of carbon dioxide	Percent- age of germina- tion of spo- rangia	pH con- centration of medium at end of experi- ment	Percentage of carbon dioxide	Percent- age of germina- tion of spo- rangia	pH con- centration of medium at end of experi- ment
Check	80	6.6	20	69	5.6
0	0	6.9	25	32	5.3
5	72	6.3	30	7	5.1
10	79	6.1	35	0	4.8
15	76	5.9			

The pH concentration of the medium was changed by different concentrations of carbon dioxide. In atmospheres containing 10 percent of carbon dioxide, the pH of the medium was changed from 6.6 to 6.1. The percentage of germination produced at this concentration is very near that produced at pH 5.8, which was obtained by a known normality of hydrochloric acid in normal atmosphere. Unlike oxygen the higher percentages of carbon dioxide produced greater acidity of the medium. This is in accordance with results obtained by Kendall (3).

HIGH AND LOW TEMPERATURES

The thick-walled sporangia of *Physoderma zae-maydis* are apparently not injured to any extent by the high summer temperatures and the cold, sometimes freezing, temperatures occurring in Florida. Eddins (2) found that sporangia stored in soil and leaf sheaths and exposed to the weather for 3 years remained viable and were capable of infecting over 50 percent of the corn plants inoculated. Tisdale (6) reported that some sporangia germinated after being allowed to freeze in a cake of ice for several days.

In an experiment conducted to determine the effect of high temperature upon the viability of sporangia, the sporangia were placed in Syracuse dishes without any water and placed in electrically controlled dark ovens held at constant high temperatures, as noted in table 5. Other sporangia were placed in Syracuse dishes containing water, which were placed in a refrigerator at 0° C. and allowed to freeze.

At 2-, 15-, and 30-day intervals, a number of the dishes were removed from the refrigerator and dark ovens. Water was added to them, and they were placed on a laboratory table at room temperature (30° C.) to germinate.

It was found that the sporangia are tolerant of constant high and low temperatures (table 5); they even germinated after being exposed to temperatures of 70° C. and 0° C. for 30 days.

TABLE 5.—The effect of high temperature and freezing on the germination of the sporangia of *Physoderma zae-maydis*

Days exposed	Percentage germination after exposure to—					
	0° C.	40° C.	50° C.	60° C.	70° C.	80° C.
2.....	86	86	86	85	84	64
15.....	45	51	46	45	42	0
30.....	7	40	37	35	28	0

SUMMARY

The effect of certain environmental factors on the germination of the sporangia of *Physoderma zae-maydis* Shaw was studied, from 300 to 500 sporangia collected from infected corn plants being used. The sporangia were placed in Syracuse dishes containing distilled water and were subjected to various controlled environmental conditions. A preliminary experiment had shown that the sporangia germinated equally well in tap water, distilled water, and corn-leaf extract.

Sporangia germinated almost equally well when exposed in sealed and nonsealed containers, indicating that a continuous exchange of fresh air is not essential for germination. They were found to germinate best in a moist chamber on a laboratory table when they received light from a north window. They also germinated well in a dark chamber under artificial light but did not germinate at all in total darkness. Direct sunlight appeared to be lethal.

In distilled water adjusted to pH 2.5 the sporangia did not germinate. The optimum reaction was pH 7.4, at which 88 percent germination was obtained.

When stored in laboratory bottles for 1, 2, and 3 years sporangia germinated to the extent of 70, 46, and 0 percent, respectively. However, previous investigations had shown that sporangia stored in laboratory bottles for 3 years produced 41 percent infection when sprayed on corn plants in the field.

Sporangia did not germinate in the absence of oxygen but germinated to the extent of 80 percent in the presence of 20 percent oxygen, which is the same percentage germination as that recorded for normal atmosphere. In the atmosphere containing 20 percent oxygen a pH value of 7.6 was produced in distilled water that had had a pH value of 6.9 at the beginning of the experiment.

Germination was best in atmospheres containing 10 percent of carbon dioxide, being 79 percent—only 1 percent less than that of the control. The pH value of the medium was changed from 6.6 to 6.1 in this case.

The sporangia were tolerant of high temperatures. They even germinated after being exposed to temperatures of 0° and 70° C. for 30 days, though there was no germination after 15 days, or after 30 days' exposure to a temperature of 80°.

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A TYPE OF LABORATORY SILO AND ITS USE WITH CROTALARIA¹

By W. M. NEAL, *associate in animal nutrition*, and R. B. BECKER, *specialist in dairy husbandry, Department of Animal Husbandry, Florida Agricultural Experiment Station*²

INTRODUCTION

In regions where the usual forage crops are poorly adapted and climatic conditions render haymaking uncertain, the use of new crops and methods for their preservation must be further investigated. Various species of the genus *Crotalaria* have been found to be well adapted culturally to the lighter sands of the Coastal Plains region (16)³ and are comparable in composition to the other legumes used in livestock feeding. The success attained at the Florida station in ensiling soybeans (5), the seasonal distribution of rainfall (17) which makes natural curing of hay an unsatisfactory practice, and the independence of silage-making of weather conditions, suggested the desirability of determining the ensilability of the crotalarias.⁴ Preservation of forage as silage has the further advantages of economy of labor and storage space (9), reasonable efficiency of conservation of nutrients and succulence, and an increase in palatability brought about by the ensiling process.

The experiments were planned to test the merits of a type of silo which was developed for this work. Four of the silos were constructed so that several species of *Crotalaria* might be studied concurrently with a minimum expenditure of materials and labor, preparatory to more exact studies of digestibility and feeding value.

WORK OF OTHER INVESTIGATORS

The first report in American literature of the ensiling of forage in experimental quantities with determinations of nutrient losses was made by Jordan (14) at the Pennsylvania station in 1884. Water-tight tubs 6 feet in height and 3 feet in diameter were filled with chopped sorghum and weighted to secure packing.

Cooke (7) reported the use of tubs 3 feet in height and 2 feet in diameter, holding about 350 pounds of maize, at the Vermont station in 1889. A layer of roofing paper and a wooden disk weighted with stones to equal 50 pounds per square foot prevented all surface spoilage. A loss of 4.98 percent in total weight and 14.67 percent in dry matter was observed. Hills (13) later made a satisfactory silage from sugar beets and straw in these silos.

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² Grateful acknowledgment is made to Will T. Dunn for analytical services and to P. T. Dix Arnold for conducting the palatability tests.

³ Reference is made by number (italic) to Literature Cited, p. 623.

⁴ The forage ensiled was provided under the terms of a cooperative agreement between the Division of Forage Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, and the Agronomy and Animal Husbandry Departments of the Florida Agricultural Experiment Station. G. E. Ritchey was in charge locally for the Division of Forage Crops and Diseases.

The early work at the Wisconsin station with silage (12, 24) was done with rectangular silos, 7 by 8 feet and 14 feet deep, with a capacity of approximately 10 tons of forage. Clover was ensiled successfully with losses of 15.4 percent of dry matter and 12.7 percent of crude protein. Lawes and Gilbert (15) had ensiled clover as well as meadow grass in the pit silos at Rothamsted with smaller losses of nutrients.

A series of studies of the adaptation of many of the forage crops for making silage was instituted at the Missouri station by Eckles (8, 10) in 1915. The final results were tabulated by Ragsdale and Turner (21). Cypress-stave silos 6 feet tall and 3 feet in diameter were filled with corn, oats and cowpeas, cowpeas, soybeans, alfalfa, Sudan grass, wheat, oats, rye, and sunflowers. All but rye made good silage although it was recommended that the legumes be ensiled only when they had wilted sufficiently to give a 30 to 35 percent dry-matter content. A wire screen was placed in the silo when it was within 18 inches of being full. The silos were sealed with a felt-lined wooden disk and weighted with 1,000 to 1,500 pounds of rock. The weight and composition of the forage placed under the screen as compared with the same data for the silage, were used to measure the unavoidable losses in the ensiling of the crops.

Reed and Fitch (22) studied the ensilage of alfalfa in upright silos 7 feet in diameter and 16 feet high, made from 1-inch flooring. Alfalfa alone made poor silage. The addition of carbohydrates in the form of meal, molasses, or sorghum increased the acid production and improved the quality. Swanson and Tague (25, 26), using quart milk bottles as containers, made parallel studies with alfalfa and sweetclover. Carbohydrate additions were beneficial and sweetclover was more desirable than alfalfa for making silage. True, Woll, and Dolcini (27) encountered no difficulty in making good silage from alfalfa alone in a commercial silo. This could be taken to indicate that conditions in the experimental silos only approximated those in larger silos.

Westover and Garver (28) ensiled 150 to 200 pounds of alfalfa, sweetclover, corn, sorghum, Sudan grass, wild sunflowers, Russian thistles, and corn or sorghum mixed in equal parts with alfalfa, in oil barrels, reheading the barrels after they were filled. Neither the sunflower nor thistle silage was palatable.

Blish (6) utilized galvanized-iron cylinders 23 to 27 inches in diameter and 7.5 feet high in his studies of factors affecting the quality of sunflower silage at the Montana station. A good-quality silage was produced except with sunflowers low in sugars. Peterson, Hastings, and Fred (20) likewise used iron cylinders 4 feet in diameter and 8 feet high, when investigating the changes that take place in the making of silage.

Woodman and Amos (1, 2, 29, 30, 32) used wood-stave silos 6 feet high and 3 feet in diameter in a series of investigations with English forage crops. The forages included oats and vetch, maize, sunflowers, sugar-beet tops, and a mixture of oats, vetch, and beans. Metal extensions were placed on the silos during filling. The silage was covered with a layer of earth to pack it and to decrease spoilage. Sample bags buried in each silo at two different levels were used for the determination of changes in composition and losses of nutrients.

Losses with the mixed silages in the experimental silos were comparable to those in commercial silos (1, 29, 33) and were smaller than those in either clamp (3) or stack (31) silage.

Even with the comparability of losses between the experimental silos and commercial silos as found by Woodman and Amos with mixed silages, and as found by Eckles (8) with maize, the complete comparability of the two types of silos may be questioned. Eckles found the legumes should have a dry matter content of 30 to 35 percent for successful ensilage in the experimental silos, while a dry-matter content of 25 percent with soybeans was found sufficient at the Florida station (18). However, all the evidence indicated the value of the laboratory silo as a tool with which to study the ensilability of forage crops.

EXPERIMENTAL METHODS AND MATERIALS

Four small pit silos were constructed in a hillside having good surface drainage. They were 43 inches in inside diameter without any taper, and between 74 and 81 inches in depth, with smooth concrete walls, and clay and limerock bottoms.

Each silo was filled with one of the crotalarias on September 22-23, 1932. The forage was cut in 1½-inch lengths in a silage cutter, weighed in tubs, and packed in the silo by tramping and tamping. When the top of the silo was reached, a piece of burlap was placed over the surface, additional cut material was packed over it, and finally the whole was covered with approximately 2 feet of earth. This earth layer was cone-shaped to carry off the rainfall. (See fig. 1.) The silos and manner of filling were designed to simulate as closely as possible the conditions found in large silos.

A 10-kg sample of the forage in a muslin bag was buried about midway in depth in the silo as it was being filled. This was covered by a thin layer of crotalaria, over which a moist piece of burlap was placed as a marker. A 1-kg sample representative of the forage in the muslin bag was taken for analysis.

The silos were opened on December 12, 1932, 81 days after they were filled. The earth and silage were stripped off to the burlap

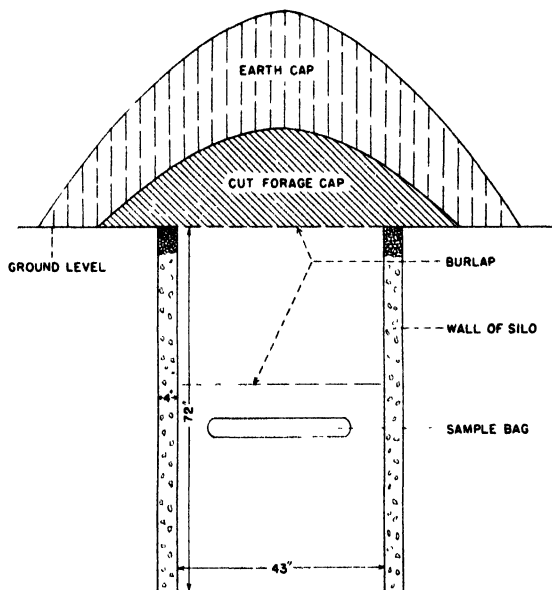


FIGURE 1. —Cross section of laboratory silo when filled

cover. Spoiled silage underneath the burlap was removed and weighed. Good silage was removed, weighed, and offered to cattle over a 16-day period to determine its palatability. The small bags were weighed as they were recovered, and sampled for analysis.

Analyses were made by the methods of the Association of Official Agricultural Chemists (4). Juice for the hydrogen-ion determinations was obtained by pressing samples of the fresh silage, and the determinations were made with a quinhydrone electrode. Losses of nutrients were calculated from the weight and composition of the forage placed in and the silage removed from the sample bags.

Four species of *Crotalaria* were used to test the feasibility of these laboratory silos and the ensilability of the forages, namely, *C. spectabilis* Roth, *C. striata* DC., *C. intermedia* Kotschy, and *C. incana* L. All were further advanced in growth than is usually considered desirable for hay, with flowers and many seed pods present. Many of the lower leaves of *C. spectabilis* and *C. incana* had fallen.

PRESENTATION OF DATA

The quantity of forage ensiled, the quantity of spoiled and sound silage recovered, and the density of the silage at each foot in the silo for each species, are presented in table 1. It will be observed that from 2,088 to 2,609 pounds of forage were cut, weighed, and packed in the silos, and from 47 to 241 pounds of spoiled silage were removed from beneath the top piece of burlap 81 days later when the silos were opened. From 1,854 to 2,329 pounds of sound silage were removed during the next 16 days. The forage over the burlap was not weighed but was estimated at 500 pounds. Over the *Crotalaria striata* silo much of this material was unspoiled, but over the others it did not keep.

TABLE 1.—*Crotalaria* forage ensiled and recovered as silage in laboratory silos, together with weights per cubic foot of the settled silage

[Forage ensiled Sept. 22-23, 1932, silos opened Dec. 12, 1932]

Species of <i>Crotalaria</i>	Forage ensiled	Silage		Depth of silo	Depth of silage	Silage density per cubic foot				
		Spoiled	Sound			First foot	Second foot	Third foot	Fourth foot	Fifth foot
	Pounds	Pounds	Pounds	Inches	Inches	Pounds	Pounds	Pounds	Pounds	Pounds
<i>C. spectabilis</i>	2,609	233	2,227	80.50	58.0	40.75	42.86	44.16	44.72	45.44
<i>C. striata</i>	2,420	47	2,322	76.00	54.5	36.55	40.09	43.15	43.92	44.16
<i>C. intermedia</i>	2,526	131	2,329	80.75	67.0	36.20	38.09	39.28	40.70	42.80
<i>C. incana</i>	2,088	241	1,854	74.00	53.0	38.71	40.52	41.17	41.53	-----

* Gravitational moisture evident.

The changes that took place in the forage were determined by comparing the weight and composition of the forage ensiled with the weight and composition of the silage recovered in the sample bags. The composition of the forage and silage, and the percentage recovery of the various constituents of the forage ensiled are presented in table 2.

TABLE 2.—Composition of fresh *Crotalaria* forage and *Crotalaria* silage, and percentage recovery of the nutrients of the forage in laboratory silos

CROTALARIA FORAGE

Species of <i>Crotalaria</i>	Net weight of material	Moisture	Dry matter	Composition of the dry matter					pH values
				Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	
	Kilograms	Percent	Percent	Percent	Percent	Percent	Percent	Percent	
<i>C. spectabilis</i>	10 000	79.14	20.86	6.89	10.00	41.70	40.03	1.38	-----
<i>C. striata</i>	10 000	74.08	25.92	3.89	17.03	39.14	37.90	2.04	-----
<i>C. intermedia</i>	10 000	75.00	25.00	5.24	12.04	41.00	40.08	1.64	-----
<i>C. incana</i>	10.000	75.68	24.32	5.81	11.40	46.40	35.12	1.27	-----

CROTALARIA SILAGE

<i>C. spectabilis</i>	9.946	81.84	18.16	7.50	10.18	46.29	33.95	2.08	4.66
<i>C. striata</i>	9.432	76.58	23.42	4.43	14.20	44.39	33.74	3.24	5.50
<i>C. intermedia</i>	9.835	77.64	22.36	5.73	10.74	46.80	33.82	2.91	5.50
<i>C. incana</i>	9.312	76.46	23.54	6.63	9.94	47.92	33.08	2.43	5.04
(Corn silage).....									3.47

PERCENTAGE RECOVERY OF THE CONSTITUENTS

<i>C. spectabilis</i>	99.46	102.85	86.59	94.25	88.11	96.12	73.44	130.51	-----
<i>C. striata</i>	94.32	97.50	85.22	97.05	71.06	96.65	75.87	135.35	-----
<i>C. intermedia</i>	98.35	101.81	87.96	96.19	78.47	100.41	74.23	156.08	-----
<i>C. incana</i>	93.12	94.08	90.13	102.85	78.59	93.09	84.90	172.46	-----

The *crotalaria* forage ensiled in the laboratory silos was found to have undergone the typical changes encountered in large silos with other crops. The destruction of protein was not so great, but the losses of dry matter and nitrogen-free extract were greater than had been obtained previously by the writers (18) with soybeans. The increase of ether extract was more marked. There had been some leaching of soluble ash. The silage had a pungent acid odor, and hydrogen-ion measurements showed it to be acid, although less so than corn silage, which was found to have a pH value of 3.47.

Quantities of the different silages were offered to growing dairy heifers to determine the relative palatability. All four of the *crotalaria* silages were placed in compartments of the same feed bunk, and the order changed from day to day. Records taken were limited to notations on order of choice and approximate amount refused.

Crotalaria incana and *C. intermedia* silages were eaten immediately and with almost no waste. *C. spectabilis* was eaten after the first two had been taken, and without significant refusal.⁵ The *C. striata* silage was refused almost completely by all of the animals (14 head) used in this study.

⁵ Unpublished work indicates a toxic principle in the seeds of *C. spectabilis* when eaten by chickens. The forage also may be poisonous, but the limited quantities of the silage offered caused no visible symptoms of poisoning. Further toxicity studies are in progress.

DISCUSSION OF RESULTS

This investigation was planned to determine whether or not such laboratory silos as are described could be used in preliminary studies with forages, and also to ascertain whether or not any of the *crotalaria*s might be desirable as silage crops. Such an investigation was necessary because the climatic conditions in Florida favor the preservation of forages as silage rather than as naturally cured hay.

The silos and the manner of filling them were designed to simulate the conditions in large silos with a minimum expenditure of labor and materials. The use of the pit type of silo, which could be capped easily with additional forage and earth, was an important feature. Leakage of air through the walls was minimized and the weight of the cap of forage and earth aided in packing the silage. How well this was accomplished is shown by the density of the silage, in table 1. The surface foot was of a density equivalent to corn silage at the eighth foot (11), or of soybean silage at the fifth foot (5). The loss by spoilage of the *crotalaria* forage actually placed in these silos was very small.

If the changes in composition and the recovery of nutrients in the laboratory silos were equivalent to those found with other crops in large silos, it could be concluded that a typical silage had been produced. If such a silage were produced, tests of its palatability would determine whether or not additional investigation of the feeding value of the silage was justified.

The demonstration in commercial practice that good silage may be made from the legumes alone, the indifferent success with alfalfa in experimental stave silos at the Kansas station (22), and the probable necessity of a dry-matter content of 30 to 35 percent in legumes for ensilage at the Missouri station (8) would indicate that commercial conditions had not been achieved in those types of experimental silos used in testing the ensilability of legumes. Chemical studies of the nutrient losses in the Kansas investigation were not presented, but the legumes of a higher dry-matter content at the Missouri station underwent normal changes and suffered the expected losses of nutrients.

In the laboratory silos filled with *crotalaria* the loss of dry matter was only slightly greater than that found with corn silage (19, 20, 23) and soybean silage (18) in commercial silos. The recovery of crude protein was good, and the increase in ether extract indicated that the fermentations resulting in ether-soluble acids had occurred, as found in normal ensilage. These results were secured with forage containing from 20.9 to 25.9 percent of dry matter from which silages containing 18.2 to 23.5 percent of dry matter were obtained. The silage had a pungent acid odor, and from both physical appearance and chemical composition, could be considered typical.

The palatability studies showed three of the four species to be much more desirable as silage than as either green forage or hay.⁶ Not only were they eaten readily, but the amounts refused were insignificant. *Crotalaria striata* has not seemed to be adapted as a livestock feed, and careful toxicity studies of *C. spectabilis* are necessary. Final selection of a particular species for ensilage must depend on additional tests with other species, as well as on the consideration of

⁶ Unpublished material from another phase of the cooperative investigation

yields, freedom from insect pests and diseases, and similar matters of an agronomic nature. All of the species studied apparently underwent typical ensilage changes to a degree that would be expected in large silos. The possible use of *C. incana* and *C. intermedia* is indicated definitely. However, they should not be recommended without additional investigation.

The potential place of a slightly larger silo of the type used in this experiment, with a capacity sufficient to supply silage for digestibility studies is worthy of consideration.

SUMMARY AND CONCLUSIONS

Typical silage was produced in laboratory pit silos of 2,000 to 2,500 pounds capacity with *crotalaria* forage. Chemical changes and losses of nutrients were of the same order as usually are encountered in large silos with other crops. Conditions more nearly comparable to those in large silos were secured than appear to have been obtained heretofore by others working with small experimental silos of other kinds in the ensiling of legumes.

Crotalaria incana and *C. intermedia* forages made palatable silage which was eaten readily by cattle. *C. spectabilis* silage also was eaten readily, but its possible toxicity requires further investigation. *C. striata* does not seem to be adapted for use as a livestock feed.

Even though some species of the genus *Crotalaria* may be ensiled with only reasonable losses and the resulting product may be palatable to cattle, there yet remains the testing of other species in the manner herein described, the consideration of cultural characteristics of the various species, and an evaluation of *crotalaria* silage as a livestock feed, before any recommendations can be made concerning the use of *crotalaria* as silage.

The potential place of a larger laboratory silo of the type used in these experiments is suggested.

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THE VITAMIN C CONTENT OF COMMERCIALY CANNED SAUERKRAUT PRODUCED UNDER KNOWN CONDITIONS¹

By HELEN T. PARSONS, *associate professor*, and CAROLYN HORN, *industrial fellow*,
*Department of Home Economics, Wisconsin Agricultural Experiment Station*²

INTRODUCTION

In a previous paper³ the antiscorbutic value of six brands of commercially canned sauerkraut was shown to vary widely. The vitamin C content of two of these brands compared very favorably with specially fermented fresh raw sauerkraut tested by Clow, Marlatt, Peterson, and Martin,⁴ while the poorest brand contained from one half to one third as much vitamin C as the best brands. Because of the lack of uniformity in the methods used in the manufacture of the sauerkraut tested, it was not possible to determine what factor or set of factors was responsible for the loss of antiscorbutic vitamin in the poorer brands. The cooperation of 4 of the 6 commercial packers who had contributed to the previous experiment was welcomed, therefore, in producing samples of canned sauerkraut under controlled conditions, as nearly uniform as feasible in all four factories. The packers who had produced the two highest scoring brands, A and B, the previous year, did not share in the present experiment.

Earlier observation⁵ that bulk sauerkraut, as purchased, may be very poor in antiscorbutic potency was confirmed by the experiments of Lawrow and Jarussowa⁶ who tested a market lot of sauerkraut which they kept in a wooden keg. The juice fed was prepared by first draining off and discarding the brine, and then squeezing the chopped sauerkraut in a cloth by hand. Thirty cubic centimeters of this juice fed daily gave complete protection against scurvy in guinea pigs, but 20 cubic centimeters gave only partial protection. The author estimated that the sauerkraut itself, without the brine, contained about 70 percent of the concentration in the juice tested.

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² Thanks are due to Dr. W. H. Peterson, Department of Agricultural Chemistry, and Dr. E. B. Fred, Department of Agricultural Bacteriology, for valuable assistance in planning the research, to W. I. Berg and other members of the research committee of the National Kraut Packers' Association for outlining the detailed procedures to be followed in the manufacture of the sauerkraut and securing the cooperation of the four sauerkraut packers, and to the packers themselves for their courtesy in supplying the data on the manufacturing procedures used. Thanks are also due Dorothy Adgate and Corrine Hogden for assistance in the preparation of the solutions, in calibration, and in chemical determinations. All solutions were checked by the senior author.

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PLAN OF EXPERIMENT

As it was desirable that the details of the method employed in producing the canned sauerkraut should be in accordance with the best commercial practice, an outline of the processes necessary to be controlled in the experiment was drawn up by members of the Departments of Home Economics, Agricultural Chemistry, and Agricultural Bacteriology of the University of Wisconsin and submitted to a research committee of the National Kraut Packers' Association for their decision as to the exact procedure to be followed. Specific detailed instructions were then sent to each of the four factories. The points covered in the instructions are indicated by the details under item in table 1. Machine canning, which presumably would give the most nearly accurate control possible in commercial practice, was employed by three of the plants. The fourth plant, however, was particularly interested in hand packing, and inasmuch as large quantities of commercial sauerkraut are packed by hand, it was thought that the inclusion of this method would make the samples more nearly representative of commercial sauerkraut and furnish a valuable control. Except for this one variation in the method of canning, an attempt was made to secure the highest degree of uniformity possible throughout the four factories.

TABLE 1.—*Condensed reports of the four sauerkraut companies on methods employed in manufacturing and canning the brands used in present experiments*

CUTTING

Item	Month	Brands*			
		C ₁	E ₂	F ₁	D ₂
Variety of cabbage used.....		All Seasons.....	All Seasons.....	Believed to be mostly Glory of Enkhuizen.	All Seasons 70 percent. All Head Glory of Enkhuizen.
Salinometer readings at time of filling tank.		15° F.....	(November 15° F March, 19° F.....)	15° F.....	(November, 16° to 15° F. April, 13°.....)
TEMPERATURE					
Room temperatures during fermentation.....	(November..... December..... January..... February..... March.....)	68°.....	72° to 48° F..... 61° to 38° F..... 38° to 30° F..... 53° to 30° F..... 62° to 54° F..... 42° to 44° F.....	53° to 70° F (range for entire time, 41° to 56° F.....)	56° to 44° F..... 63° to 46° F..... 38° to 36° F..... 62° to 48° F..... 39° to 50° F..... 44° to 46° F.....
Temperature of the cabbage at time of cutting.		Estimated to be 45° to 50° F	56° to 48° F..... 48° to 44° F..... 43° to 30° F..... 40° to 30° F..... 49° to 44° F..... 45° to 49° F.....	70° to 61° F..... 70° to 60° F..... 68° to 53° F..... 65° F.....	56° to 44° F..... 35° to 48° F..... 34° to 42° F..... 34° to 46° F..... 33° to 30° F..... 31° F.....
Temperature of sauerkraut during fermentation.		55° F. (estimated)			
Temperature of cold sauerkraut at time of canning.		46° F.....			

* Listed in order of vitamin C concentration.

TABLE 1—Condensed reports of the four sauerkraut companies on methods employed in manufacturing and canning the brands used in present experiments—Continued

ACIDITY

Item	Month	Brands			
		C ₂	E ₂	F ₂	D ₂
Average acidity of fermenting sauerkraut for each month, expressed as lactic acid.	November December January February March				
Acidity of the bulk sauerkraut as it goes into the can.		2.16 percent	1.46 percent ^a	1.66 percent	Percent
Total acidity, expressed as percentage lactic acid, in the canned sauerkraut (average of 20 determinations for each brand).		1.98 percent ^b	1.46 percent ^b	1.40 percent	Percent
Highest reading of acidity in cans ^a		1.49 percent	1.22 percent	1.43 percent	0.72
Lowest reading in acidity in cans ^a		1.45 percent	1.10 percent	1.37 percent	0.68
Reduction in acidity during canning		28 percent	22 percent	16 percent	1.25
pH of canned sauerkraut ^a		5.2	5.2	5.2	1.24
					1.31
					1.52
					1.54
					1.66
					1.67
					1.69
					1.72
					1.76

CANNING

Length of fermentation.	121 days.	130 days.	92 days.	145 days.
Date of opening tank and canning.	March 6.	March 17.	February 19.	April 2.
Time in preheating tank.	Estimated to be 6 minutes.	Approximately 20.	4 minutes.	10 minutes.
Temperature of sauerkraut as it leaves preheating tank and is put into cans.	180° F.	132°-137° F.	110° F.	130° F.
Method of packing.	Machine.	Machine.	Hand.	Machine.
Additional brine or water used in canning.	Mixture of about 3 barrels salt water to each 15 barrels of sauerkraut in preheating vat.	Mixture of 5 percent brine and 95 percent pure water added in automatic filler.	Hot salt brine (not sauerkraut brine) in preheating vat.	Approximately 3 ounces water added to each can in the filler.
How the amount of brine added is determined.		To control acidity (tests at various intervals).	Added to keep up the heat.	

Time in exhaust.....	5 minutes.....	5 minutes.....	7 minutes.....
Temperature in the can before sealing.....	140° F. bottom..... 140° F. center..... 184° F. outside.....	120° F. center.....	140° F. center..... 184° F. outside.....
Cooking after sealing.....	None.....	Cooked in rotary Anderson and Harngrover continuous rotary cooker for 10 minutes; center of can after cooking was 165°.....	None.....
SOLID AND LIQUID CONTENTS OF COMMERCIALY CANNED SAUERKRAUT			
Average weight of the drained solid content of sauerkraut in 16 or more cans of each brand.....	681 grams.....	675 grams.....	717 grams.....
Average weight of liquid in 16 cans of each brand.....	103 grams.....	131 grams.....	89 grams.....
Additional liquid required to completely fill cans of sauerkraut: A, range of the six determinations made for each brand.....	28 to 55 cubic centimeters..... 38 cubic centimeters.....	28 to 46 cubic centimeters..... 39 cubic centimeters.....	25 to 56 cubic centimeters..... 42 cubic centimeters.....
Reduced pressure in cans of sauerkraut, average of three determinations for each brand.....	8 pounds.....	12 1/2 pounds.....	37 to 60 cubic centimeters..... 43 cubic centimeters..... 12 1/2 pounds.....

^b Acidity tests made at University of Wisconsin. Average acidity of C₁ was 2.07 percent.

^c Figures for last determination of acidity in fermentation tank.

EXPERIMENTAL PROCEDURE

The details of the care of the experimental animals and the feeding of the doses of sauerkraut were identical with those employed by Clow, Parsons, and Stevenson.⁷

Standard solutions and calibrated glassware and thermometers were furnished to the four companies by the Department of Home Economics of the University of Wisconsin.

The hydrogen-ion content of the canned sauerkraut was determined by means of a Leeds and Northrup potentiometer with a quinhydrone electrode.

Total acidity was determined by titrating with N/10 sodium hydroxide 10 cc of sauerkraut juice after the addition of 15 cc of water and 1 cc of a 1 percent solution of phenolphthalein and after a few seconds of boiling.

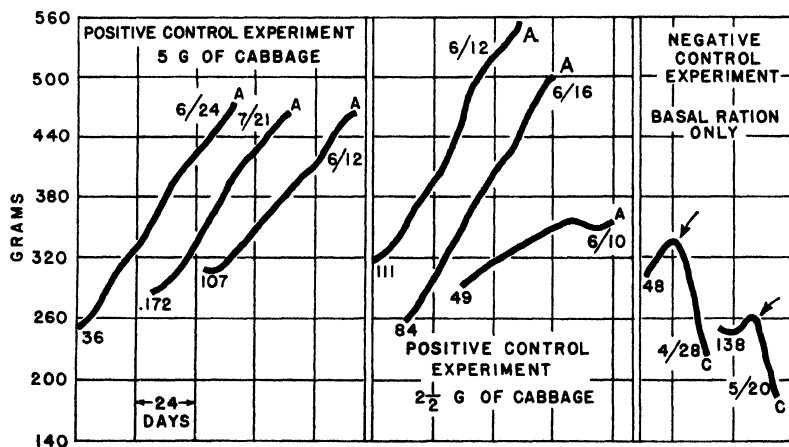


FIGURE 1.—Control experiments on vitamin C. In the positive control experiment, the animals received 5- or 2½-g doses of raw cabbage daily. In the negative control experiment, the animals were fed the basal ration alone; arrows above the weight curves indicate the point at which pronounced scurvy became evident. The month and day of the month on which autopsy was performed are indicated near the end of the weight curve of each animal. Autopsy symbols are as follows: A, no sign of scurvy; A?, possibility of scurvy; A-, slight but definite signs of scurvy; B+, moderate scurvy; B-, C+, and C, increasing degrees of scurvy.

Determinations of the solid contents of the cans of sauerkraut were made as follows: Each can of sauerkraut, upon being opened, was rapidly spread on a 9 by 4 inch piece of wire gauze of ¼-inch mesh, suspended in a metal pan into which the juice drained. The total weight of sauerkraut and juice was determined during the 2-minute period allowed for the juice to drain. At the end of this period, which was determined by a time clock, the wire gauze containing the sauerkraut was removed from the pan, and the weight of the juice alone was ascertained. The weight of the total solids was then determined by difference.

RESULTS

Each of the four brands of sauerkraut tested in these experiments is identified in this report by the same letter assigned to it in the study carried out previously⁷ and an inferior figure 2 is added to indicate the

⁷ CLOW, B., PARSONS, H. T., and STEVENSON, I. See footnote 3.

second series of experiments in the present study. The sequence of letters therefore indicates the rank of the brands in the relative concentration of vitamin C in the first study, but not, in each case, in the second study.

In carrying out the set of instructions in the four factories, some unavoidable variations occurred because of such incidents as failure of the sauerkraut to attain the required acidity in an expected time in certain of the tanks. On the whole, however, the processes of manufacture were decidedly more nearly uniform in the four factories than for the six brands tested during the preceding year, and much more detailed information was secured in the present experiment. The data for the manufacture of the four brands of sauerkraut are given in table 1.

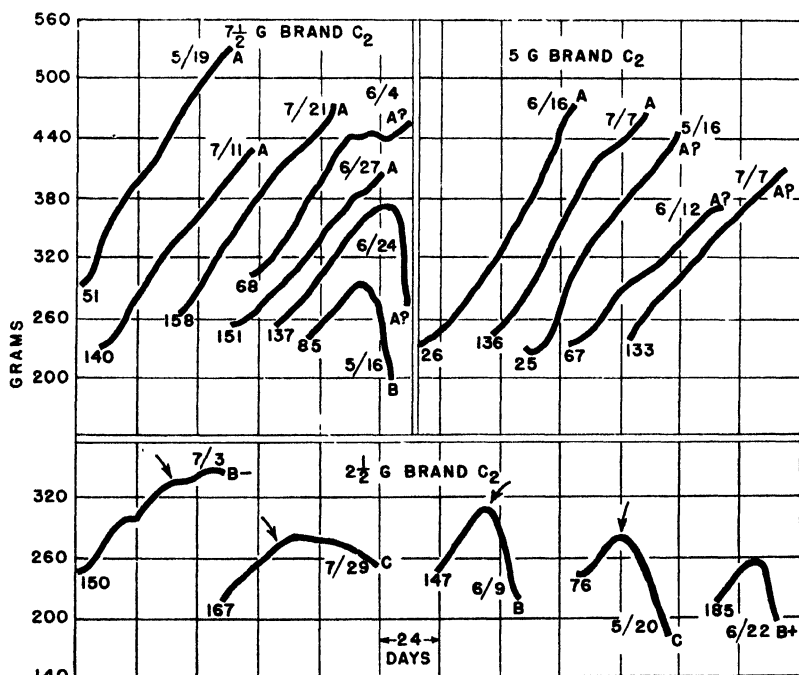


FIGURE 2.—Growth of guinea pigs and degree of protection from scurvy afforded them by $7\frac{1}{2}$ -, 5-, and $2\frac{1}{2}$ -g daily doses of commercial sauerkraut, brand C_2 . For explanation of symbols see figure 1.

One hundred and thirty-one guinea pigs were used in the experiment, but only 80 are included in this report. No arbitrary selection was made of the data, however, inasmuch as all animals are included except those which were discarded for the following reasons: Failure to eat the daily portion of sauerkraut; refusal to eat the basal ration, with resultant starvation; and the presence of intercurrent abnormalities such as infected lungs or liver. In a few instances such as no. 137, figure 2, and nos. 83, 88, and 92, figure 4, the growth curves indicate some abnormality not connected with a deficiency of vitamin C, but since no specific pathological condition was detected on autopsy, the records of these animals were retained in order to make the report entirely impartial.

A problem arose in connection with attempting to secure quantitative intake of the 10- and $7\frac{1}{2}$ -g doses of brands E₂ and D₂, respectively, not heretofore encountered. In former experiments, the only pronounced cases of scurvy occurred on relatively small doses of sauerkraut where quantitative intake was easy to secure. In the present experiment the two brands mentioned were found to have such a low vitamin C content that it proved impossible, except in five instances, to induce quantitative intake of the 10- or $7\frac{1}{2}$ -g daily portions after scurvy became pronounced. At the onset of scurvy the gums and

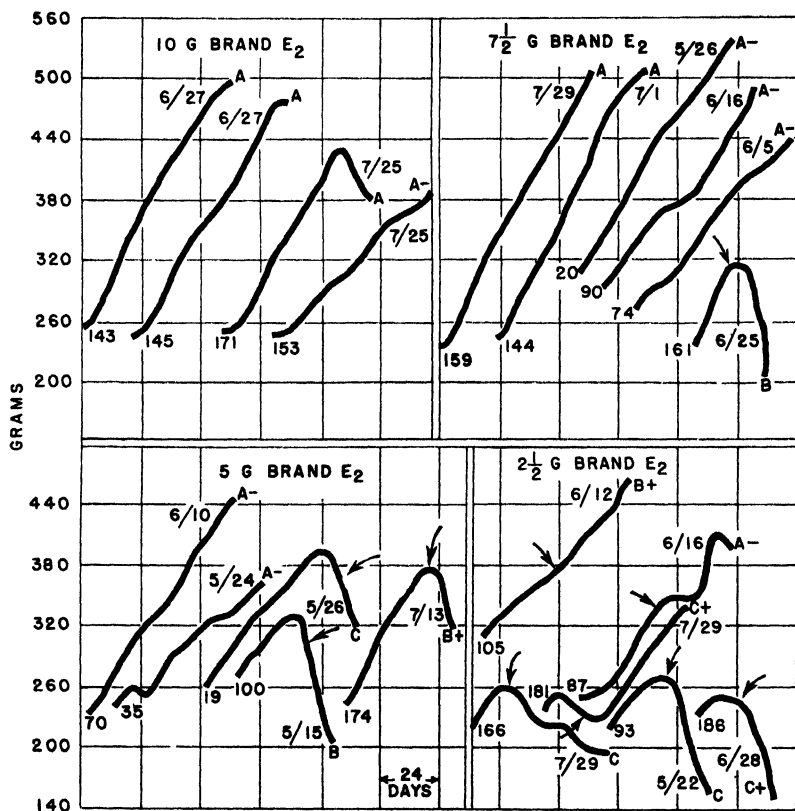


FIGURE 3.—Growth of guinea pigs and degree of protection from scurvy afforded them by 10-, $7\frac{1}{2}$ -, 5-, and $2\frac{1}{2}$ -g daily doses of commercial sauerkraut, brand E₂. For explanation of symbols see figure 1.

teeth of guinea pigs usually become tender and painful, and as a result both the total food intake and the body weight decline, as a rule. In all cases where the consumption of the daily dose of sauerkraut became irregular before the onset of scurvy, the animals were discarded. But in the case of the guinea pigs on large doses of these two brands of sauerkraut it was necessary to include the records of all animals which consumed their doses quantitatively up to the time of the appearance of severe scurvy, and to indicate the intervals of irregular intake by broken lines on the graphs. The results of the feeding experiments are presented in figures 1 to 5, inclusive.

CONTROL EXPERIMENTS

In the negative control experiment where only the basal ration was fed, both of the animals developed signs of pronounced scurvy in 16 days. The first signs of incipient scurvy occurred in 7 days.

In the positive control experiment, the six animals fed the basal ration with the addition of either 5 or $2\frac{1}{2}$ g of cabbage daily were fully protected from scurvy and, except in the case of no. 49, responded with excellent growth.

The growth curves and incidence of scurvy of guinea pigs on the $7\frac{1}{2}$ -, 5-, and $2\frac{1}{2}$ -g portions of sauerkraut brand C₂ per day (fig. 2) were practically identical in this study, in which the sauerkraut was produced under controlled conditions, and that made previously under the routine procedures of the factory⁸. Protection from scurvy

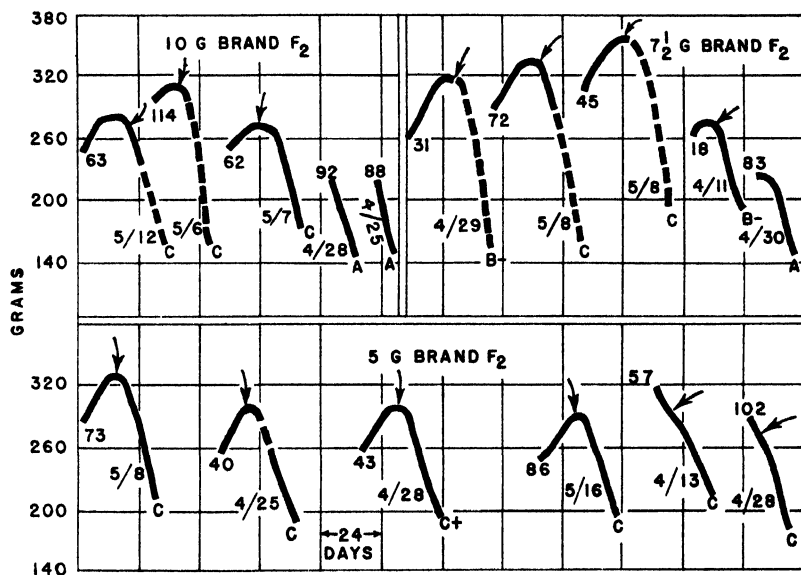


FIGURE 4—Growth of guinea pigs and degree of protection from scurvy afforded them by 10-, $7\frac{1}{2}$ -, and 5-g daily doses of commercial sauerkraut, brand F₂. Broken lines indicate periods of failure to eat quantitatively the doses offered. For explanation of symbols see figure 1.

was somewhat better on the $7\frac{1}{2}$ -g level in last year's experiment in comparison with the present results, but was correspondingly slightly poorer on the 5- and $2\frac{1}{2}$ -g levels.

The protection from scurvy afforded by brand F₂ is approximately the same as for brand E from the same factory, tested previously. The results on the higher doses are slightly inferior in the present experiment but are better on the $2\frac{1}{2}$ -g level.

The results of the present experiments on brands F₂ (fig. 4) and D₂ (fig. 5) compare very unfavorably with those obtained in the former study on brands D and F from the same factories. In the records presented in figures 4 and 5 there is almost no indication to be found of the presence of vitamin C in the sauerkraut, even at a

⁸ CLOW, B., PARSONS, H. T., and STEVENSON, I. See footnote 3.

level of 10 g daily. By a comparison with the negative control experiment in figure 1, it may be seen that scurvy occurs, on an average, as soon when these brands of sauerkraut, at even $7\frac{1}{2}$ - or 10-g levels, are fed daily with the basal ration, as when the basal ration is fed alone. In the case of guinea pigs 83, 88, and 92 which were fed brand F_2 and which did not develop scurvy, death ensued so quickly from some unknown cause that the symptoms of scurvy would not have become evident in so short a time had the animals been fed the basal ration only.

No data were secured for a $2\frac{1}{2}$ -g daily dose of brand F_2 inasmuch as the negative results of the higher levels were available before this dose was started, and it was quite evident that it would be superfluous to feed the lower amount.

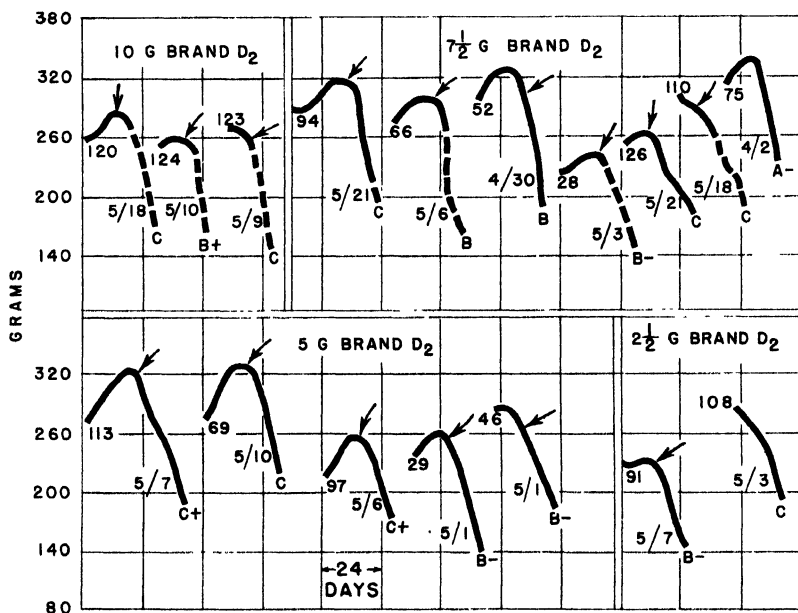


FIGURE 5. Growth of guinea pigs and degree of protection from scurvy afforded them by 10-, $7\frac{1}{2}$ -, 5-, and $2\frac{1}{2}$ -g daily doses of commercial sauerkraut, brand D_2 . Broken lines indicate periods of failure to eat quantitatively the doses offered. For explanation of other symbols see figure 1.

Although no difference can be seen in the degree of failure to protect from scurvy between brands F_2 (fig. 4) and D_2 (fig. 5), the latter is given a lower rating in the list because of the greater difficulty in inducing the guinea pigs to eat this brand, and the number of failures of animals on it for unexplained reasons. Brand D_2 was also the only one in which some of the cans showed dark streaks in the sauerkraut and even swells and leakage during the course of the experiment. These cans were, of course, rejected, and the material used for feeding was only from cans of good appearance.

DISCUSSION

Of the 4 brands of commercial sauerkraut produced under known, and somewhat uniform conditions, 2, brands C_2 and E_2 , were found to contain a significant amount of vitamin C. Indeed, the richer of

these 2, brand C₂, is fairly comparable in antiscorbutic value with the specially fermented raw sauerkraut tested by Clow, Marlatt, Peterson, and Martin.⁹ Furthermore, these 2 commercial brands duplicated very closely the antiscorbutic value of the sauerkraut manufactured the previous year by the same two packers, respectively, under their routine factory conditions.¹⁰ The other 2 brands, on the other hand, showed no demonstrable antiscorbutic potency even when fed at a 10-g level. Consumption of larger doses by the guinea pigs was not obtained, but these experiments show that if these 2 brands contain any antiscorbutic vitamin it is at least less than one fourth the amount in the 2 richer brands.

In view of the striking differences found in the vitamin C content of the 4 brands, it is of interest to compare the details of manufacturing procedure in the different plants as they are listed in table 1.

The variety of cabbage is identical only in the two richer brands. While no experiments have been reported on variations in vitamin C content of different samples of fresh raw cabbage, it is known that variety is a factor in the vitamin C content of certain fruits. Bracewell, Hoyle, and Zilva¹¹ have shown that one variety of English cooking apple is outstandingly superior in vitamin C content to all the other varieties tested by them. However, if All Seasons cabbage were strikingly richer in antiscorbutic vitamin than the other varieties used in these experiments, it would be expected that brand D₂, containing 70 percent of this variety, would be more nearly like brands C₂ and E₂ in antiscorbutic potency than like brand F₂. In regard to length of fermentation, temperature of fermenting vat, degree of acidity, and processes of canning, there seems to be no one factor common to both brands F₂ and D₂ as compared with brands C₂ and E₂, to account for the differences found between these two groups. It is known that vitamin C is particularly sensitive to conditions of low acidity or of alkalinity in the presence of heat. However, brand E₂, which had an outstandingly low acidity in the fermentation vat and in the can, was 1 of the 2 richer brands. Again, these 2 richer brands were the ones subjected to the greatest dilution by water or salt brine during canning, as indicated by the percentage of reduction in acidity between the fermenting vat and the sealed can. It is true that the average unfilled space in the cans of the richer brands was slightly less than in the other 2, but the degree of variation among individual cans of all 4 brands makes this appear of little significance as a gage of possible oxidation within the can after sealing.

It would seem that the differences in vitamin C content of the 4 brands must be attributable to some unidentified factor or factors which were not controlled in this experiment, but which are perhaps dependent on the regular procedure in manufacture in the different plants, inasmuch as 2 of the brands studied practically duplicated their former vitamin C content, and another brand which was previously at the bottom of the list was 1 of the 2 in which no vitamin C was demonstrated in the present experiment.

⁹ CLOW, B., MARLATT, A. L., PETERSON, W. H., and MARTIN, E. A. See footnote 4.

¹⁰ ———, PARSONS, H. T., and STEVENSON, I. See footnote 3.

¹¹ BRACEWELL, M. F., HOYLE, E., and ZILVA, S. S. THE ANTISCORBUTIC POTENCY OF APPLES. *Biochem Jour.* 24: 82-90 1930.

SUMMARY

A study of 4 brands of commercial sauerkraut manufactured under known and fairly comparable conditions showed that 2 of these brands practically duplicated their previous antiscorbutic content, when observed under routine conditions of the factory. The other 2 brands did not show any antiscorbutic potency when fed at even a 10-g level, a record much inferior to previous results on the same brands, although 1 of these brands made an especially poor record, also, in the previous test.

No satisfactory correlation can be traced between the comparative vitamin C content in the four individual brands on the one hand, and variations in processes of manufacture on the other.

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FUSARIUM RESISTANCE IN WISCONSIN HOLLANDER CABBAGE¹

By MELVIN E. ANDERSON²

Formerly agent, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and fellow in plant pathology, University of Wisconsin

INTRODUCTION

The cabbage-yellows disease, caused by *Fusarium conglomerans* Wr., has been important in the Middle West for the last 3 decades. An investigation of the disease was begun at the Wisconsin Agricultural Experiment Station in 1910 (3).³ From this study it was concluded that the only practical way of controlling the disease was through the development of resistant strains of cabbage (*Brassica oleracea* L.). From two plants of the Danish Ballhead or Hollander variety that remained healthy throughout the season of 1910 on soil thoroughly infested with the yellows organism, continued mass selection was conducted until a resistant strain or variety, known as "Wisconsin Hollander", was secured and introduced for general use in 1916 (4). In the early trials Wisconsin Hollander proved to have a high degree of resistance as compared with commercial varieties (3). At the end of the season of 1914 the proportion of healthy plants in the F₂ progenies was from 90 to 100 percent. In 1916 the disease was unusually destructive (4). Several fields of Wisconsin Hollander showed a high incidence of the disease, and the average percentage of diseased plants for 20 fields was 24.3. The majority of affected plants, however, were but slightly diseased and succeeded in forming heads. This high percentage of diseased plants was probably caused by the severe weather conditions in 1916. Later field observations have likewise indicated that Wisconsin Hollander may show a high percentage of diseased plants under severe conditions of high soil temperature, which is often accompanied by relatively low soil moisture.

Greenhouse tests have shown that Wisconsin Hollander seedlings grown in soil infested with the causal organism differ in degree of resistance (6). Resistance to the disease was found to be broken down under high controlled temperatures. Walker and Smith (9) compared Wisconsin Hollander with progenies of single plant selections from other varieties at various temperatures in infested soil. They found that the resistance of Wisconsin Hollander to yellows was

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² The writer wishes to express his appreciation to Dr. J. C. Walker, of the University of Wisconsin, under whose direction this work was conducted, for helpful suggestions and criticisms, and to Dr. R. A. Brink, also of the University of Wisconsin, for suggestions regarding the genetical phases of the study.

³ Reference is made by number (italic) to Literature Cited, p. 661.

quite different from that exhibited by homozygous resistant lines developed by Walker (7) and by Walker et al. (8). The latter remained perfectly healthy in infested soil at constant soil temperatures as high as 26° C. Wisconsin Hollander showed quite a high percentage of diseased plants at 20°, with little or no resistance to the disease at 26°. Walker (7) showed that in his homozygous resistant lines resistance was governed by a single dominant gene. He suggested the possibility of hereditary factors being present, which may act quite independently of the main gene for resistance and may modify the expression of the disease. An appreciable percentage of plants of mass-selected varieties, such as Wisconsin Hollander, were suspected of containing such factors.

The object of the investigation reported herein was to study the nature of resistance of Wisconsin Hollander cabbage to the yellows disease in comparison with that displayed by homozygous resistant lines.

MATERIAL AND METHODS

Four types of cabbage host material were used in this study. Wisconsin Hollander seed was secured from various commercial sources. For convenience the individual lots were labeled WH-1, WH-2, WH-3, WH-4, and WH-5.⁴ Seeds shown by various field and greenhouse tests to be homozygous resistant and homozygous susceptible were obtained from J. C. Walker. The homozygous resistant lines were as follows: 40-28-A and 40-29-A, selected from All Head Early; 20-29-A, from Jersey Wakefield; 21-30-A, from Copenhagen Market; and 33-31-B, from Danish Ballhead. The homozygous susceptible line used was C-29-A, selected from Copenhagen Market. Two commercial varieties were used, namely, Danish Ballhead, or Hollander, and a Flat Dutch type known as Smith Pride. Both of these lots, when tested, were found to contain from 95 to 100 percent of susceptible individuals.

Methods used in selecting and handling plants for seed propagation have been discussed by previous writers (8). For genetical studies, plants were grown to seed in the greenhouse during the winter, where controlled pollination was practiced throughout. All flowering branches were covered with paper bags to reduce contamination. Field tests were carried out on thoroughly infested soil in Racine and Kenosha Counties, Wis., where similar tests had been made for a number of years. The trial plot was examined at frequent intervals throughout the summer. Each diseased plant was marked with a bamboo stake as a permanent seasonal label.

Greenhouse tests under controlled temperature conditions were carried out mainly in artificially inoculated soil. Inoculum prepared by growing a single-spore isolation of the yellows organism on a mixture of corn meal and sand was thoroughly mixed with soil that had been steamed for a period of 4 hours at a pressure of 15 pounds. Several lots of susceptible seedlings were grown on the inoculated soil in order to ascertain the thorough distribution of the fungus before it was used in the trials reported herein. In a few tests both inoculated soil and naturally infested soil were used. Inoculated soil was found

⁴Although these commercial stocks were presumably derived from the original stock introduced in 1916, it was expected that they would differ from one another after having been propagated for a period of years in the hands of different seedsmen. The difference in degree of resistance is discussed later.

to insure more nearly uniform and more severe tests because of the greater amount and better distribution of the fungus. In naturally infested soil the disease developed less evenly and the test for resistance was prolonged. Wisconsin soil-temperature tanks were employed to maintain constant soil temperatures. The moisture content of the soil was maintained at about 60 percent of the moisture-holding capacity.

FIELD TESTS OF COMMERCIAL WISCONSIN HOLLANDER CABBAGE

The degree of success with Wisconsin Hollander on infested soil is largely dependent on environmental conditions. Under ordinary field conditions a high percentage of plants remain healthy, but in unusually warm midsummer weather 50 percent may show symptoms of the disease (4, 7). During 1929, 1930, and 1931, observations and tests were made in the field. Table 1 shows the results of some of these tests in comparison with commercial Hollander⁵ and resistant pure lines. The hot dry weather in the summer of 1931 was very favorable to a severe attack by the yellows organism. In one field in Dane County, near Madison, Wis., 67 percent of the Wisconsin Hollander plants counted were diseased and 19 percent were dead from yellows. In the same field 97 percent of the commercial Hollander plants were diseased and 79 percent were dead from yellows. There were small areas in the Racine commercial field where no diseased plants were found, probably because of incomplete infestation of the soil by the fungus. These two fields were more severely diseased than commercial fields in general, but the data show how Wisconsin Hollander may react under environmental conditions very favorable for the disease.

TABLE 1. - Occurrence of cabbage yellows in Wisconsin Hollander, in homozygous resistant lines, and in commercial susceptible lines in the field in Wisconsin, 1930 and 1931

Strain or variety	Location	Year	Total plants		Diseased plants
			Number	Percent	
WH-5	Experimental plot (Kenosha County)	1930	121		43
40-20-A ^a	do	1930	157		0
Commercial Hollander	do	1930	289		98
WH-1	Experimental plot (Racine County)	1931	160		52
WH-2	do	1931	30		77
WH-3	do	1931	38		37
WH-4	do	1931	112		97
40-28-A ^a	do	1931	130		0
Commercial Hollander	do	1931	286		98
Wisconsin Hollander	Commercial field (Dane County)	1931	1,192		67
Commercial Hollander	do	1931	370		97
Wisconsin Hollander	Commercial field (Racine County)	1931	1,430		59

^a Homozygous resistant line.

In trials made in 1930 on the Kenosha experimental plot with seed lot WH-5 (table 1) 43 percent of the plants were diseased. The four lots of Wisconsin Hollander tested in 1931 on the Racine experimental plot differed considerably in percentage of diseased plants. Some

⁵ Two names, Danish Ballhead and Hollander, are sometimes used interchangeably for this variety. It will be referred to herein as "commercial Hollander."

differences may be expected when seed produced in widely separated localities is used. When seed of a mass-selected variety is produced on soil free from the yellows organism, plants very susceptible to the disease are not eliminated. Seed from continued propagation on such soils would therefore be expected to give a higher percentage of susceptible plants than seed from repeated propagation on soil infested with the yellows organism. Such a history is suspected for the seed lot WH-4, which gave a very high percentage of diseased plants when tested on infested soil.

Under ordinary field conditions the majority of diseased Wisconsin Hollander plants show but incipient yellows and may be classed as slightly diseased. Such plants usually recover and may succeed in forming heads. It is common for such plants to show slight symptoms during the warmest part of the summer and to recover later with the recurrence of cooler weather. Visible symptoms may be confined to one or two of the lower leaves, and as these leaves drop from the plant no further sign of the disease is seen.

F₁ PROGENIES OF SELECTED WISCONSIN HOLLANDER PLANTS

FIELD EXPERIMENTS

For genetical studies, 55 Wisconsin Hollander plants were selected at random, in 1929, from four lots, namely, WH-1, WH-2, WH-3, and WH-4, growing on soil free from the disease organism, and placed in the greenhouse for seed propagation during the winter. Each plant was self-pollinated, and crosses were made with pollen from homozygous resistant and homozygous susceptible lines. Forty three of these plants produced seed.

The F₁ progenies were tested in the field during the summer of 1930. Duplicate plantings of progenies from self-pollination and from the susceptible crosses were made in soil free from the yellows organism, in order to assure material for further genetic studies. On October 29, final notes were taken and all plants of certain progenies were saved and placed in cold storage. The results of field tests with F₁ progenies are presented in table 2. The families are arbitrarily divided into four classes based on the percentage of diseased plants appearing in the progenies from self-pollination.

The progenies from self-pollination showed various degrees of susceptibility and resistance, ranging from 100 percent diseased to 100 percent healthy. The majority of the progenies tested proved to be very high in percentage of plants susceptible to yellows. Twenty-four progenies showed from 75 to 100 percent of the plants diseased (table 2, class 1), and 13 progenies of this class were totally diseased. There was also considerable variation within the individual progenies of this class. In some progenies, such as 8-33s⁶ and 8-47s, the plants succumbed completely and comparatively early in the season; in other progenies, such as 8-23s and 8-30s, a large percentage of affected plants were but slightly diseased and some of these recovered; that is, they ceased to show visible symptoms of the disease by the end of the season. The progenies of class 2 and of class 3 showed, respectively, increases in degree of resistance to the disease. A majority of affected plants in these classes were but slightly diseased, and in only

⁶ The small letter "s" following a number denotes a progeny from self-pollination.

one progeny was there more than one plant dead of yellows. Two of the progenies tested (class 4) remained entirely healthy throughout the season. Progeny 8-11s appeared to be the most promising in resistance and yield; of the 42 plants of this line, 41 formed heads of good shape and size.

TABLE 2.—Occurrence of cabbage yellows in the 1930 field tests with F_1 progenies from selected Wisconsin Hollander, commercial Wisconsin Hollander, commercial Hollander, and a homozygous resistant line

Variety and classification ^a	Parent plant no.	Progenies from self-pollination		Progenies from crosses with --			
				Homozygous susceptible lines		Homozygous resistant lines	
		Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants
		Number	Percent	Number	Percent	Number	Percent
WH (selection)·	8-1	42	100	22	100	—	—
	8-2	52	98	50	100	25	0
	8-3	45	100	48	100	24	0
	8-5	25	100	49	100	—	—
	8-7	27	100	42	100	—	—
	8-8	26	100	36	100	14	0
	8-10	22	100	—	—	—	—
	8-12	42	88	54	100	32	0
	8-13	42	95	61	100	25	0
	8-16	29	83	64	98	—	—
	8-23	49	80	60	97	24	0
	8-27	25	100	71	100	26	0
	8-30	54	76	49	80	9	11
	8-32	36	97	45	96	27	0
	8-33	21	100	61	100	26	0
	8-34	25	100	74	92	25	0
Class 1 (75 to 100 percent of self-pollinated plants diseased)	8-36	17	94	60	95	26	4
	8-41	44	100	59	100	29	0
	8-43	31	94	54	100	19	0
	8-45	47	98	54	100	26	0
	8-46	37	100	57	100	31	0
	8-47	49	100	38	100	26	0
	8-51	47	94	47	94	75	0
	8-52	46	100	54	98	68	0
	8-25	21	62	71	99	27	0
	8-38	57	42	64	81	29	0
Class 2 (25 to 75 percent of self-pollinated plants diseased)	8-40	57	61	54	82	26	0
	8-42	12	50	81	65	26	0
	8-53	37	32	59	93	58	0
	8-4	47	19	49	65	—	—
	8-9	26	27	41	80	27	0
	8-14	36	8	48	38	25	0
Class 3 (1 to 25 percent of self-pollinated plants diseased)	8-15	34	18	66	67	26	0
	8-17	51	4	—	—	—	—
	8-19	50	18	54	96	27	0
	8-26	58	14	53	74	—	—
	8-28	47	6	61	23	26	0
	8-37	17	12	64	58	25	0
Class 4 (no disease in self-pollinated plants)	8-39	63	21	29	79	26	0
	8-11	42	0	41	61	28	0
	8-49	26	0	73	49	27	0
WH-5 (commercial)	—	121	43	—	—	—	—
Commercial Hollander	—	289	98	—	—	—	—
Homozygous resistant	—	157	0	—	—	—	—

^a For convenience the parent plants are arbitrarily divided into four classes based on the percentage of diseased plants appearing in the progenies from self-pollination.

For comparison with these progenies from self-pollination of selected Wisconsin Hollander plants, Wisconsin Hollander (WH-5), commercial Hollander, and a homozygous resistant line were also tested. Most of the plants of the commercial Hollander were dead of yellows at the end of the season. Forty-three percent of the commercial Wisconsin Hollander were diseased. Of these, 21 percent

were severely diseased or dead and 22 percent but slightly diseased. No symptoms of the disease appeared in plants of the homozygous resistant line.

Except 2 plants of 987 tested, the progenies from crosses of selected Wisconsin Hollander $\times$ homozygous resistant plants remained perfectly healthy throughout the season. In view of the fact that the pistillate mother plants of the two progenies in question gave very susceptible progenies from self-pollination, it is suspected that the two diseased plants were the result of chance self-pollination. The progenies from crosses with homozygous susceptible plants were in general very susceptible to the disease. Not only were the percentages of diseased plants high, but the majority of such plants were dead or yellows or severely diseased.

Certain F_1 progenies were tested in the field in 1931. Homozygous resistant plants and commercial Hollander were also planted for comparison. Environmental conditions were more favorable for the disease during this season and the percentage of diseased plants was much higher throughout. Progenies from crosses with homozygous susceptible lines, which had exhibited some resistance in 1930 (table 2, class 3), showed a considerable increase in percentage of diseased plants in 1931. The homozygous resistant line and the commercial Hollander reacted identically as in 1930 (table 3).

TABLE 3.—Occurrence of cabbage yellows in 1931 field tests of F_1 progenies from selected Wisconsin Hollander, commercial Hollander, and a homozygous resistant strain

[Data represent the combined results of plantings on two separate dates]

Parent plant no. or variety	Progenies from self-pollination		Progenies from crosses with homozygous susceptible plants	
	Total plants	Diseased plants	Total plants	Diseased plants
Wisconsin Hollander (selection).	Number	Percent	Number	Percent
8-4	29	72	60	93
8-11	48	8	39	62
8-14	45	62	44	75
8-23	45	93	15	100
8-28	22	50	34	85
8-30	31	87	70	89
8-33	87	100	67	100
8-37	42	55	28	86
8-39	53	53	65	91
8-40	24	88	15	100
8-47	22	100	56	100
8-53	20	70	61	95
Commercial Hollander	286	98		
Homozygous resistant	130	0		

GREENHOUSE EXPERIMENTS

REACTION AT RELATIVELY HIGH SOIL TEMPERATURES

Studies with Wisconsin Hollander under conditions of controlled soil temperature were planned to supplement the field tests. The most resistant F_1 progenies from self-pollinated Wisconsin Hollander plants and from crosses with susceptible plants were tested in October and November in comparison with homozygous resistant and homozygous susceptible lines (table 4). The tests were continued for 25

to 29 days at a soil temperature of about 22° C. and an air temperature of about 20° C. All the F_1 progenies from self-pollination showed a high percentage of diseased plants and some were totally diseased. The majority of diseased plants in these progenies were dead of yellows at the end of the test. The 8-4s strain, however, showed more resistance than in the field trials. The progenies from the susceptible crosses exhibited very few plants free from the disease, and most of the diseased plants were dead at the end of the experiment. In these tests, the susceptible cross with 8-49, which showed some resistance in the 1930 field trials (table 2), proved to be more susceptible than the susceptible cross with 8-11. The commercial Hollander and the homozygous susceptible strain tested succumbed completely to the disease, whereas the homozygous resistant strain remained perfectly healthy.

A similar experiment, with commercial Wisconsin Hollander and the most resistant F_1 progenies from self-pollination of selected Wis-

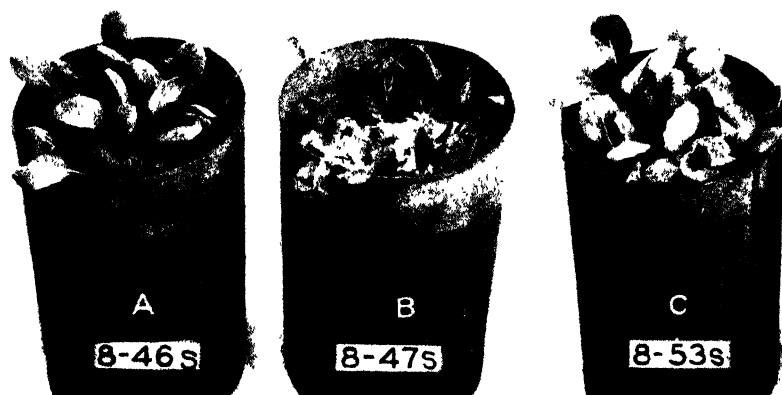


FIGURE 1 Comparison of the rate of disease development in three susceptible F_1 progenies from Wisconsin Hollander plants grown on infested soil at 20° to 22° C. for 21 days. Many of the plants of 8-46s and 8-53s show incipient yellows, and all the plants of 8-47s have succumbed to the disease. At the end of 36 days most of the plants of 8-46s and 8-53s were dead of yellows.

consin Hollander plants, was conducted in May, when the air temperature was slightly higher than in the previous tests. In this test the progenies showed a higher percentage of diseased plants than in the first experiment.

The more susceptible F_1 progenies from self-pollination when similarly tested showed little or no resistance. All plants in some progenies died comparatively early, whereas plants in other progenies were uniformly slower to succumb. Figure 1 illustrates the differences in results obtained from progenies showing slightly different reactions to the disease.

In another experiment four F_1 progenies that had previously shown various rates of disease development in field and greenhouse were grown at a series of constant soil temperatures. The four strains represented the most susceptible progeny (8-33s), two intermediate progenies (8-46s and 8-39s), and the most resistant progeny (8-11s) from self-pollination of Wisconsin Hollander plants. The percentage

of diseased plants at various intervals and the condition of the plants at the end of the experiment are shown in table 5.

TABLE 4.—Occurrence of cabbage yellows in the most resistant F_1 progenies from selected Wisconsin Hollander, commercial Hollander, a homozygous resistant line, and a homozygous susceptible line, when grown at a constant soil temperature of 22° C. in inoculated soil

Parent plant no. or variety	Progenies from self-pollination		Progenies from susceptible crosses	
	Total plants	Diseased plants	Total plants	Diseased plants
Wisconsin Hollander (selection)	Number	Percent	Number	Percent
8-4	38	53	47	98
8-9	40	80		
8-11	40	73	30	90
8-14	40	100	35	100
8-15	40	93	45	100
8-17	40	90	33	100
8-19	40	93		
8-26	40	80	45	98
8-28	40	78	45	100
8-37	40	100	37	100
8-39	40	100		
8-49			40	98
Commercial Hollander	30	100		
Homozygous susceptible	47	100		
Homozygous resistant	48	0		

TABLE 5.—Development of cabbage yellows in certain F_1 progenies from selected Wisconsin Hollander plants when grown in inoculated soil at various constant soil temperatures

Soil temperature	Strain no.	Total plants	Plants diseased after—						Plants in indicated condition after 21 days			
			6 days	8 days	11 days	14 days	19 days	21 days	Healthy	Slightly diseased	Severely diseased	Dead of yellows
		Number	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
16° C.	8-33s	39	0	0	21	33	61	80	20	29	51	0
	8-46s	43	0	0	0	9	14	51	49	42	9	0
	8-39s	37	0	0	0	5	8	46	54	35	11	0
	8-11s	27	0	0	0	4	4	11	89	11	0	0
18° C.	8-33s	37	0	0	54	84	100	100	0	0	49	51
	8-46s	45	0	0	18	36	58	100	0	53	47	0
	8-39s	37	0	0	19	32	76	88	12	45	40	3
	8-11s	28	0	0	0	11	11	32	68	21	11	0
20° C.	8-33s	39	0	18	77	100	100	100	0	0	8	92
	8-46s	45	0	0	31	80	91	100	0	13	25	62
	8-39s	38	0	0	47	87	95	100	0	8	29	63
	8-11s	20	0	0	19	42	58	62	38	31	19	12
22° C.	8-33s	36	8	70	100	100	100	100	0	0	0	100
	8-46s	44	0	0	52	95	100	100	0	2	18	80
	8-39s	37	0	0	70	73	100	100	0	3	16	81
	8-11s	31	0	0	16	58	74	74	26	41	23	10
24° C.	8-33s	46	22	94	100	100	100	100	0	0	0	100
	8-46s	42	0	17	93	100	100	100	0	0	2	98
	8-39s	40	0	15	93	100	100	100	0	0	3	97
	8-11s	27	0	11	78	93	96	100	0	7	19	74
26° C.	8-33s	43	28	88	100	100	100	100	0	0	0	100
	8-46s	45	0	18	100	100	100	100	0	0	0	100
	8-39s	35	0	37	100	100	100	100	0	0	0	100
	8-11s	25	0	20	92	96	100	100	0	4	4	92

Both in percentage of plants diseased and in the condition of the diseased plants, the severity of the disease increased in all strains with the rise in soil temperature. Even the most resistant strain became totally diseased at temperatures above 22° C. This strain (8-11s) showed a relatively high degree of resistance to yellows at 16° and 18°, the majority of affected plants at these temperatures being but slightly diseased. The most susceptible strain (8-33s), however, showed little or no resistance to yellows at the soil temperatures maintained. The relative degrees of resistance of the four strains at the end of 14 days are shown in figure 2. At the end of the experi-

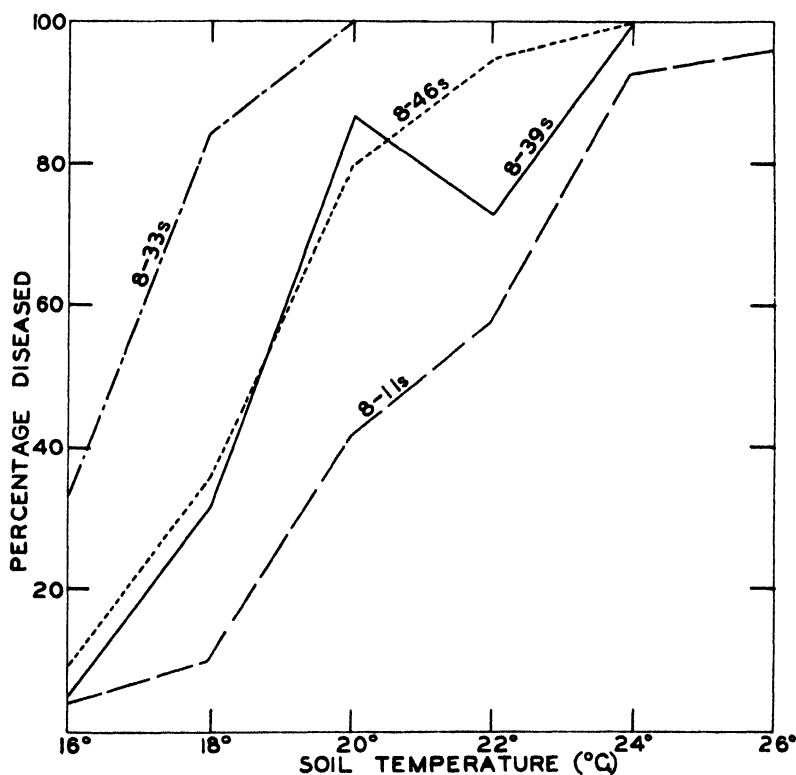


FIGURE 2.—Comparison of four F_1 progenies from Wisconsin Hollander plants at the end of 14 days when grown in inoculated soils at various constant soil temperatures (See table 7)

ment the relative degrees of resistance were apparent only at the lower temperatures. At the higher temperatures all strains were totally diseased. The incubation period was consistently shorter in the more susceptible strains. The homozygous resistant lines remained completely healthy at all temperatures from 16° to 26°.

The reactions of 8-33s, 8-53s, 8-11s, and homozygous resistant 33-31-B, under similar conditions, are shown in figure 3, demonstrating the wide variation in rate and degree of disease development in the F_1 progenies from Wisconsin Hollander plants.

The results of the field tests show that the progenies from self-pollination ranged from 100 percent diseased to 100 percent healthy.

Some of the mother plants apparently transmitted little resistance to their progenies, and these were probably of the class in the Wisconsin Hollander variety which become severely diseased or succumb under environmental conditions favorable for the disease. The progenies of several mother plants contained individuals that were but slightly affected by the disease and showed a tendency to recover. The progenies of a small percentage of the plants exhibited a high degree of resistance under severe field conditions, and the majority of the diseased plants were but slightly affected. However, constant soil temperatures at 22° to 24° C. served to inhibit the expression of this resistance, whereas the resistance of the homozygous resistant lines, attributed by Walker (7) to the gene *R*, remained completely stable. This physiological test serves to distinguish between the resistance in Wisconsin Hollander and that in the homozygous resistant lines described by Walker.

Gradations in degree of disease development among the F_1 progenies were quite evident in the greenhouse tests. It was also



FIGURE 3. Comparison of three F_1 progenies from self-pollinated Wisconsin Hollander plants and a homozygous resistant line, when grown in inoculated soil at a constant soil temperature of 22° C. for 30 days. All plants of strain 8-33s are dead, all plants of strain 8-53s are diseased, and 3 have partially recovered in strain 8-11s, 2 plants have remained healthy throughout, and 7 have shown slight symptoms and are recovering. The homozygous resistant strain, 33-31-B, has remained healthy. Each can contained 15 plants.

distinctly noticeable that reaction of individuals within any one F_1 progeny was more uniform than in the commercial Wisconsin Hollander variety. The practice of self-pollination tends to increase the uniformity of the degree of resistance or susceptibility in the individuals of a given F_1 progeny as compared with that of the Wisconsin Hollander variety in general.

The degree of resistance of the mother plants, as displayed in tests with progenies from self-pollination, is considerably diminished in progenies from crosses between those mother plants and homozygous susceptible plants. When Wisconsin Hollander plants were crossed with plants homozygous for the main gene for resistance the dominance of the latter in the F_1 hybrids was complete. Walker (7) discussed resistance in homozygous resistant and homozygous susceptible lines on the basis of plants falling into two discontinuous classes. In Wisconsin Hollander, however, many intermediate classes or degrees of resistance are found. These classes differ from one another not only in the percentage of plants susceptible to the

disease but also in the severity of the disease in such plants. If the resistance exhibited by the Wisconsin Hollander variety were controlled by a single Mendelian factor, the progeny from self-pollination of a heterozygous plant would be expected to give 3 resistant plants to 1 susceptible, and the progeny from a heterozygous $\times$ susceptible cross should give 1 resistant to 1 susceptible plant. None of the F_1 progenies tested over a period of 2 years gave susceptible and resistant plants in numbers approximating these ratios. The resistance of Wisconsin Hollander, therefore, is apparently the result of factors genetically different from the single Mendelian factor of the homozygous resistant lines described by Walker (7).

REACTION OF SUSCEPTIBLE PROGENIES AT LOW SOIL TEMPERATURES

In the greenhouse studies it was observed that in certain very susceptible F_1 progenies from self-pollinated Wisconsin Hollander plants the development of disease was more rapid at low temperatures than had heretofore been observed. Experiments were planned to study this question more critically. A coverless Wisconsin soil-temperature tank was used in these experiments. A low temperature was maintained in the tank by a constant stream of cold water. The air temperature was quite constant at 22° to 24° C. Small variations of soil temperature between cans and within each can were obtained by placing the cans at various depths in the tank. The cans were placed in five rows across the tank. The soil level in the first row of cans was slightly lower than the water level; that of each succeeding row was raised in successive increments of 2 inches. The last row of cans was placed on top of the tank. With such an arrangement constant soil temperatures were maintained at a small increase in temperature in each ascending row. In each can there was a slight variation in temperature at different depths of the soil. This variation more closely simulated soil-temperature conditions in the field than does the ordinary tank method, in which the whole can is at the same temperature.

In the first experiment, progenies 8-33s and 8-46s were studied. In the field trials (table 2) both strains had shown 100 percent of the plants diseased, but the plants of strain 8-33s were more severely diseased than those of strain 8-46s. The soil temperature varied from 13.5° C. in the first row of cans (A) to 20.1° in the fifth row (E). The percentage of diseased plants was ascertained at various intervals over a period of 23 days (table 6). At the three lower temperatures there appeared to be a distinct difference between the two strains in the rate of development of the disease. Progeny 8-33s showed a high percentage of diseased plants at all temperatures, and the few plants showing but slight symptoms were confined to the cans of the two lower temperatures. Progeny 8-46s showed no symptoms in row A and but slight symptoms in rows B and C.

In the second experiment a homozygous resistant strain (33-31-B) was grown in comparison with 8-33s and 8-46s, and the test was continued for 42 days. Lower temperatures were maintained to find the critical temperature at which symptoms of the disease appear in the very susceptible strain 8-33s. Temperatures of the surface soil were noted as well as those at depths of 1 and 3 inches. The results (table 7 and fig. 4) further indicate a distinct difference in the rate of

TABLE 6.—*Development of cabbage yellows in two F_1 progenies from Wisconsin Hollander plants of different degrees of susceptibility when grown in inoculated soil at various soil temperatures*

[20 plants of 8-33s and 12 plants of 8-46s were grown in each row]

Row	Soil temperature at the depth of—		Strain no	Plants diseased after					Plants in indicated condition after 23 days		
	3 inches	1 inch		10 days	12 days	15 days	19 days	23 days	Slightly diseased	Severely diseased	Dead of yellows
	°C	°C		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
A	13.5	13.9	8-33s 8-46s	0 0	0 0	10 0	45 0	70 0	35 0	35 0	0 0
B	13.9	14.6	8-33s 8-46s	10 0	45 0	60 0	100 17	100 33	10 33	90 0	0 0
C	14.5	15.6	8-33s 8-46s	75 0	100 8	100 8	100 17	100 17	0 0	5 5	95 0
D	16.0	17.3	8-33s 8-46s	100 17	100 75	100 83	100 92	100 100	0 8	0 33	100 59
E	20.1	20.0	8-33s 8-46s	100 8	100 33	100 92	100 100	100 100	0 0	0 8	100 92

[15 plants of each strain were grown in each row]

Row	Soil temperature at the depth of—			Strain no	Plants diseased after						Plants in indicated condition after 42 days		
	3 inches	1 inch	¼ inch		8 days	14 days	21 days	29 days	38 days	42 days	Slightly diseased	Severely diseased	Dead of yellows
	°C.	°C.	°C.		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
A.....	10.9	11.9	12.3	{ 8-33S 8-46S 33-31-B	0 0 0	0 0 0	0 0 0	20 0 0	33 0 0	47 0 0	47 0 0	0 0 0	
B.....	11.8	13.4	14.3	{ 8-33S 8-46S 33-31-B	0 0 0	0 0 0	53 0 0	80 33 0	87 47 0	100 80 0	27 80 0	0 0 0	
C.....	13.1	14.8	15.6	{ 8-33S 8-46S 33-31-B	0 0 0	27 0 0	100 20 0	100 40 0	100 60 0	100 67 0	0 60 0	27 7 0	
D.....	15.5	17.0	17.2	{ 8-33S 8-46S 33-31-B	13 0 0	93 80 0	100 100 0	100 100 0	100 100 0	100 100 0	0 7 0	0 93 0	
E.....	20.8	21.3	20.9	{ 8-33S 8-46S 33-31-B	80 33 0	100 100 0	100 100 0	100 100 0	100 100 0	100 100 0	0 0 0	100 100 0	

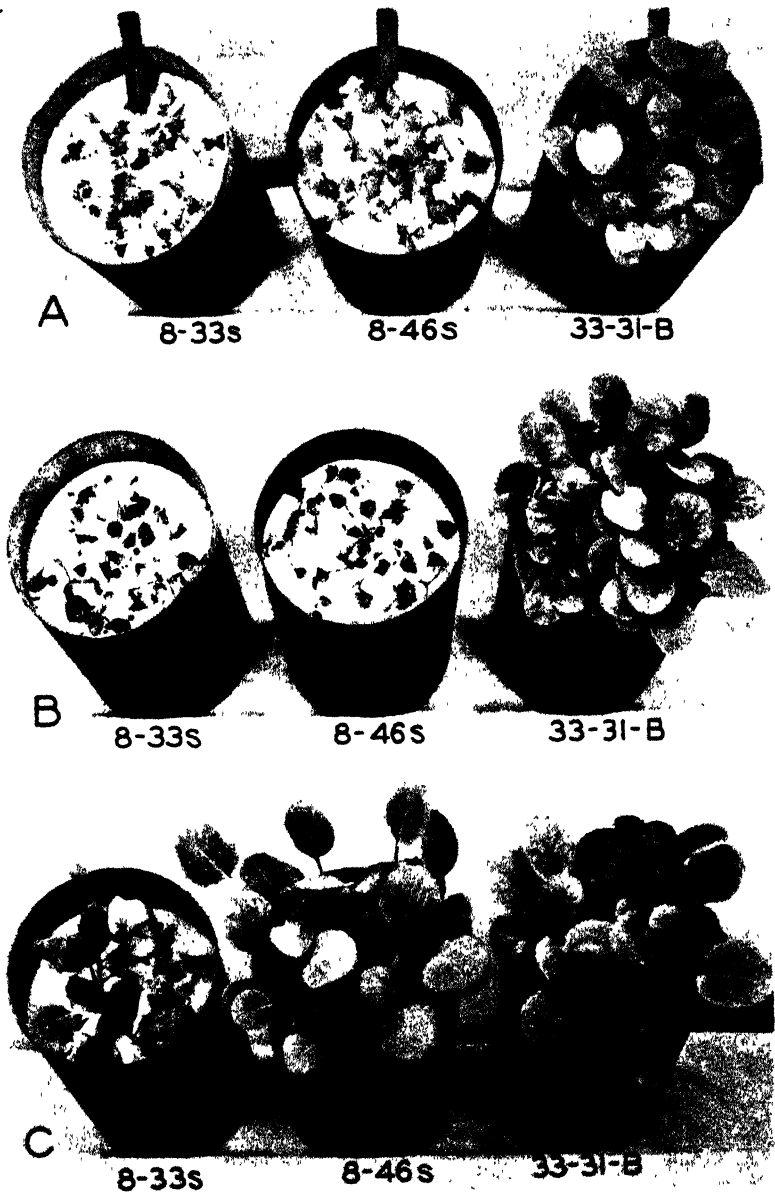


FIGURE 4.-- Comparison of two susceptible F_1 progenies, 8-33s and 8-46s, and a homozygous resistant strain, 33-31-B, when grown in inoculated soil at various low soil temperatures. Each can contained 15 plants. A, Plants after 13 days at 20.8° to 21.3° C. (table 7, row E); all plants of 8-33s and 8-46s are diseased; the majority of the latter are still alive. B, Same as A, after 36 days; all plants of 8-33s and 8-46s have succumbed. C, Plants after 36 days at 13.1° to 14.8° (table 7, row C); all plants of 8-33s are diseased and 10 are dead; 7 plants of 8-46s are slightly diseased and the remainder healthy. The homozygous resistant strain, 33-31-B, remained healthy throughout. Note that at 20° there is little difference in reaction between the two susceptible strains, whereas at the lower soil temperature there is considerable difference.

The incubation period of the disease was greatly lengthened at the lower temperatures, from 8 days at 20° to 21° C. to 29 days at 10° to 12°. The time limit must be considered at these low temperatures. In the first experiment 8-46s showed no diseased plants at 13.5° to 13.9° at the end of 23 days (table 6). In the second experiment, however, the disease occurred in this strain at the end of 29 days at 11.8° to 13.4° (table 7).

The critical soil temperature at which incipient yellows occurs in strain 8-33s is probably between 10° and 12° C. These results are quite different from those of earlier workers (2, 5, 6) who reported the approximate minimum temperature for disease occurrence at 17°. Recently Blank (1) has shown that the disease develops at soil temperatures as low as 12° to 13°. His conclusion that the critical soil temperature was near 12° is borne out in the foregoing experiments, and the evidence indicates that the critical soil temperature for the appearance of yellows symptoms may be slightly below 12° for certain very susceptible strains of cabbage.

These experiments indicate that the F_1 progenies from self-pollinated Wisconsin Hollander plants which vary slightly in reaction to the yellows disease have distinctly different degrees of resistance at low soil temperatures. The expression of this resistance is markedly dependent upon the temperature of the soil. For example, strain 8-33s is susceptible to the disease at temperatures as low as 10° to 12° C. At this temperature strain 8-46s remains healthy, but becomes susceptible to the disease at a slightly higher soil temperature. In other words, plants of different progenies differ in genetic composition with reference to number or kind of factors making for resistance. Such a hypothesis would explain the various degrees of resistance found in the different F_1 progenies from self-pollinated Wisconsin Hollander plants.

RELATION OF AGE TO RESISTANCE

The idea that the resistance of Wisconsin Hollander to the yellows disease increases with the age of the plants has been accepted for some time. This conception has arisen mainly from the work of Tisdale (6), who concluded that older plants are more resistant than younger ones and that resistance becomes more stable as the plants grow older. This conclusion was not borne out in some preliminary tests of the present study and experiments were planned to determine more definitely the relation of age to resistance in this variety.

The first experiment was conducted during November 1930 in a bench one half of which contained inoculated soil and the other half naturally infested soil. The soil temperature was kept quite constant at about 22° C. by means of steam pipes beneath the bench, but varied at times from 19° to 25°. The air temperature was about 22°. Two lots of Wisconsin Hollander (WH-1 and WH-4) and two F_1 progenies from self-pollinated Wisconsin Hollander plants (8-11s and 8-26s) were tested. Seedlings of each strain, previously grown for 30, 45, 60, and 75 days, respectively, on clean soil, were transplanted into each of the two types of infested soil. Because of the diminished sunlight during the early winter months these plants were not as large as plants grown in the spring. The test was continued in the inoculated soil for 20 days and in the naturally infested soil for 25

days. Table 8 shows the percentages of diseased plants at approximately regular intervals. The data indicate that within the limits of the experiment there were no consistent differences in degree of resistance attributable to increase in the age of the seedlings. The rate of occurrence of disease in seedlings of different ages differed but slightly in any single strain. Moreover the older seedlings were as severely diseased as the younger seedlings of the same strain. In relation to one another, the four strains reacted to the disease as in previous soil-temperature tank tests. The incubation period was longer and the disease developed more slowly in the naturally infested soil than in the inoculated soil.

TABLE 8.—*Development of cabbage yellows in two F_1 progenies from self-pollinated Wisconsin Hollander plants and in commercial Wisconsin Hollander seedlings when transplanted at various ages to inoculated or to naturally infested soil and grown at a soil temperature which varied from 19° to 25° C.*

Strain no	Age of plants when transplanted	Plants transplanted to inoculated soil						Plants transplanted to naturally infested soil					
		Total plants	Plants diseased after—					Total plants	Plants diseased after—				
			7 days	10 days	13 days	17 days	20 days		7 days	10 days	13 days	17 days	25 days
		Number	Percent	Percent	Percent	Percent	Percent	Number	Percent	Percent	Percent	Percent	Percent
S 11s	Days												
	30	29	3	24	52	83	86	21	0	5	21	43	67
	45	13	2	21	40	81	84	25	0	4	16	40	72
	60	20	0	10	40	80	80	7	0	14	14	43	71
	75	25	0	32	51	88	88	7	0	11	29	57	86
S-26s	30	25	1	28	80	88	92	18	0	6	17	61	78
	45	20	15	40	85	95	95	9	0	11	33	78	89
	60	30	7	42	63	97	97	11	0	0	27	73	91
	75	25	8	40	64	96	96	9	0	11	22	67	78
	30	58	10	53	88	98	100	28	4	7	25	71	93
W H-1	45	50	12	42	76	92	96	26	0	15	40	73	96
	60	39	8	39	72	97	97	26	0	23	69	88	96
	75	38	13	61	84	95	100	8	0	25	75	100	100
	30	58	40	90	93	98	100	20	5	25	70	90	100
	45	37	51	84	97	100	100	28	4	43	82	96	100
W H 4	60	37	32	73	92	100	100	19	16	37	78	100	100
	75	37	27	78	92	100	100	9	11	56	89	100	100

In the next test seedlings grown from seed in inoculated soil were compared with seedlings that had been grown in clean soil and then transplanted to inoculated soil. This experiment was conducted during March and April 1932 and the tests were made only at the soil temperatures of 20° and 22° C., because, as has been shown, the resistance of Wisconsin Hollander is broken down completely at higher temperatures in inoculated soil. The seeds to be planted in the inoculated soil were first placed on moist filter paper in sterile Petri dishes for 1 day to assure uniform germination. These germinating seeds were planted in inoculated soil simultaneously with seedlings of different ages that had been grown in clean soil. The seedlings were then placed at a rather low temperature (12° to 15°) for 1 day to allow partial recovery from transplanting before they were placed in the soil-temperature tanks. The cans containing the planted seeds were placed immediately in the soil-temperature tanks.

The first symptoms of disease appeared after 7 days at 22° C., and the diseased plants were noted each day thereafter. Table 9 shows

the percentage of diseased plants at various intervals. In all cases, both in this experiment and in duplicate tests, the seedlings grown from seed in inoculated soil were slower in showing symptoms of the disease than were seedlings grown on clean soil for 15 days before being transplanted to inoculated soil. When these results are compared with those of previous experiments, a similar difference in rate of disease occurrence is noted also between seedlings from seed in inoculated soil and 30-day-old transplants. The percentage of diseased plants was markedly smaller in seedlings grown from seed in inoculated soil. This was especially noticeable in the earlier disease counts. Toward the end of the tests the percentage of diseased plants tended to become equal in the seedlings of different ages. The disease appeared earlier and developed more rapidly in the susceptible strain (8-33s) in seedlings of all ages. The relative degrees of resistance of different strains were quite uniform in the seedlings of different ages.

From these results it appears that the resistance of Wisconsin Hollander is an inherent character present in the youngest seedlings, not a character acquired or developed coincidentally with the increase in age of the plant. It is possible that vigor and development may modify the expression of resistance in Wisconsin Hollander but this would probably be of minor importance in comparison with the resistance resulting from the genetical constitution of the plant. No such modification was noted in the foregoing tests. These results with young seedlings are quite different from those of Tisdale (6), but were consistent in three tests.

TABLE 9.—Comparison of resistance of Wisconsin Hollander seedlings grown from seed in inoculated soil with that of seedlings grown for various periods in clean soil and then transplanted to inoculated soil

[Plants were grown at soil temperatures of 20° and 22° C.]

Strain no.	Age of plants when transplanted	Temperature of inoculated soil 20° C.						Temperature of inoculated soil 22° C.					
		Total plants	Plants diseased after					Total plants	Plants diseased after—				
			8 days	10 days	14 days	16 days	20 days		8 days	10 days	14 days	16 days	20 days
			Number	Percent	Percent	Percent	Percent		Number	Percent	Percent	Percent	Percent
8-33s	0	42	0	48	93	100	100	30	3	70	97	100	100
	15	20	35	100	100	100	100	20	80	95	100	100	100
	48	12	0	58	100	100	100	15	27	100	100	100	100
	67	12	0	33	100	100	100	12	0	100	100	100	100
8-37s	0	35	0	9	20	43	66	40	0	13	33	67	78
	15	20	0	35	75	75	90	20	0	35	85	90	90
WH-1	0							57	0	2	28	77	88
	15	20	0	10	75	80	85	20	0	25	80	90	90

* Seed planted in inoculated soil.

F₂ PROGENIES FROM SELECTED WISCONSIN HOLLANDER PLANTS

The reaction of the F₂ progenies from Wisconsin Hollander plants indicates that this variety consists of a heterogeneous group of individuals (table 2), ranging from those highly resistant to those completely susceptible to the yellows disease under field conditions. The

F₁ progenies from the Wisconsin Hollander plants 8-4, 8-11, 8-23, 8-28, 8-39, 8-40, 8-47, and 8-49 were saved in the fall of 1930 for further genetical study. These progenies represented various degrees of resistance exhibited by Wisconsin Hollander plants, and consisted of susceptible crosses, resistant crosses, and progenies from self-pollination.

F₂ PROGENIES FROM SELF-POLLINATION

A series of progenies derived from Wisconsin Hollander plants by two generations of selfing was tested in 1931 in inoculated soil at constant soil temperatures. The first test was carried out in October and November (table 10). At the end of 30 days at a soil temperature of 22° C., many plants of some strains were still apparently healthy. Such individuals were transplanted to a bench containing inoculated soil for an additional test lasting 38 days. The soil temperature in the bench was usually 22°, but varied from 19° to 24°. At the end of this second test, plate cultures were made from all doubtful plants. Eight plants of the strain 8-149s were healthy beyond doubt after a third test at 24°. A few F₂ progenies (8-103s, 8-147s, 8-149s) were more resistant and others were more susceptible than were the parent progenies when tested under similar conditions. (Compare with tables 3 and 4.) Duplicate tests with these strains gave similar results, except that 8-146s and 8-148s showed a small percentage of healthy plants at the end of 30-day tests.

TABLE 10.—*Development of cabbage yellows in F₂ progenies from self-pollinated Wisconsin Hollander plants when grown in inoculated soil at 22° C. for 30 days and then at 19° to 24° for an additional 38 days*

Parent plant no	Strain no	Total plants	Plants diseased after—			Parent plant no	Strain no	Total plants	Plants diseased after—		
			30 days	68 days					30 days	68 days	
		Number	Percent	Percent			Number	Percent	Percent		
8-4s	8-100s	60	85	97	8-30s	8-143s	33	100	100		
	8-103s	35	29	74		8-144s	54	100	100		
	8-104s	48	54	94		8-146s	36	100	100		
	8-106s	19	38	79		8-147s	41	93	93		
8-40s	8-136s	73	100	100		8-148s	32	100	100		
						8-149s	34	76	79		

Although the number of Wisconsin Hollander plants represented by these F₂ progenies is small, the results indicate that resistance to yellows can be increased by selection in self-pollinated lines. The variability of resistance in F₂ progenies suggests the interaction of modifying factors, the reaction of a particular strain depending upon the assortment of modifying factors which it possesses. The high resistance shown by the eight plants from 8-149s is probably the result of a combination of factors very favorable for resistance. It is also possible that these plants resulted from chance contamination or error during pollination.

F₂ PROGENIES FROM SUSCEPTIBLE CROSSES

Twenty-five progenies were obtained from self-pollination of F₁ plants from the cross Wisconsin Hollander × a homozygous susceptible plant. The F₂ progeny tests were conducted in inoculated soil

in Wisconsin soil-temperature tanks, and in a bench containing inoculated soil. The soil temperature of 22° to 23° C. was maintained in the tanks, and in the bench the soil temperature was usually 22° but varied from 19° to 24°. The tests were about 30 days in duration. All progenies showed almost complete susceptibility to yellows, although the Wisconsin Hollander parent plants were of the more resistant classes (table 2). Furthermore, the majority of the F_1 parent plants had been grown on infested soil in 1930, thus representing the survivals of their respective progenies. The same degree of resistance as that of the Wisconsin Hollander parent plants was not recovered in any progeny resulting from selfing plants of the F_1 susceptible cross. Such reactions are not uncommon, however, when the character in question is controlled by modifying factors. These results indicate that the modifying factors which control resistance in Wisconsin Hollander plants are absent in the susceptible line used, and in the resultant cross these factors were dispersed or segregated so that the original resistance was not recovered in the F_2 generation.

F_2 PROGENIES FROM RESISTANT CROSSES

The F_2 progenies obtained by selfing plants from the resistant cross Wisconsin Hollander $\times$ 21-30-A were tested in inoculated soil at temperatures of 22° and 22° to 24° C. These tests are summarized in table 11. It is evident that every progeny segregated as a monohybrid, yielding approximately 25 percent of diseased plants. The deviation from the theoretical 3 to 1 ratio is practically negligible. In only one progeny was the deviation three times the probable error, and in this progeny (8-107Js) more than one fourth of the plants were diseased. Among the progenies from crosses with the different Wisconsin Hollander plants there was no apparent difference in respect to rate of disease occurrence or severity of disease in individual plants. The progenies representing the more resistant Wisconsin Hollander plants (8-11, 8-49) gave a closer fit to the 3 to 1 ratio than those representing the more susceptible Wisconsin Hollander plants (8-47, 8-23). This difference is of no significance, however, as all progenies gave a very close fit.

TABLE 11.—Occurrence of cabbage yellows in the F_2 progenies from self-pollination of the F_1 crosses of Wisconsin Hollander $\times$ homozygous resistant plants (table 2) when grown in inoculated soil at constant soil temperatures of 22° to 24° C.

Wisconsin Hollander parent no.	Progeny no.	Total number of plants		Deviation	Probable error	Dev. P. E.
		Healthy	Diseased			
8-23	8-99Js	25	8	0.3	1.7	0.2
	8-100Js	138	30	5.3	3.9	1.4
	8-101Js	84	25	2.3	3.1	.7
	8-102Js	38	10	2.0	2.0	1.0
	8-103Js	124	37	3.3	3.7	.9
	8-104Js	52	22	3.5	2.5	1.4
Total		461	141	19.5	7.2	2.7
8-28	8-105Js	83	21	5.0	3.0	1.7
	8-106Js	17	9	2.5	1.5	1.7
	8-107Js	62	34	10.0	2.9	3.5
	8-108Js	54	14	3.0	2.4	1.3
Total		216	78	4.5	5.0	.9

TABLE 11.—Occurrence of cabbage yellows in the F_2 progenies from self-pollination of the F_1 crosses of Wisconsin Hollander $\times$ homozygous resistant plants (table 2) when grown in inoculated soil at constant soil temperatures of 22° to 24° C.—Con.

Wisconsin Hollander parent no	Progeny no	Total number of plants		Deviation	Probable error	Dev. P.E.
		Healthy	Diseased			
8-39	8-115Js	65	29	5.5	2.8	2.0
	8-116Js	40	13	3	2.1	.1
	8-117Js	81	25	1.5	3.0	.5
	8-118Js	99	30	2.3	3.3	.7
Total		285	97	1.5	5.7	3
8-40	8-125Js	31	10	3	1.9	.2
	8-126Js	108	38	1.5	3.5	.4
	8-127Js	15	7	1.5	1.4	1.1
	8-128Js	85	21	5.5	3.0	1.8
Total		239	76	2.8	5.2	.5
8-47	8-129Js	4	1	3	7	.4
	8-130Js	122	32	6.5	3.6	1.8
	8-131Js	93	25	4.5	3.2	1.4
	8-132Js	155	48	2.8	4.2	.7
	8-133Js	70	25	1.3	2.9	.4
	8-134Js	138	43	2.3	3.9	.6
Total		582	174	15.0	8.0	1.9
8-49	8-135Js	77	27	1.0	3.0	.3
	8-136Js	12	7	2.3	1.3	1.8
	8-137Js	65	19	2.0	2.7	.7
	8-138Js	57	17	1.5	2.5	.6
	8-139Js	136	44	.8	3.9	.2
	8-140Js	10	2	1.0	1.0	1.0
Total		357	116	2.0	6.4	.3
8-11	8-144Js	76	20	4.0	2.9	1.4
	8-145Js	71	23	.5	2.8	.2
	8-146Js	31	10	3	1.9	.2
Total		178	53	4.8	4.4	1.1
Grand total		2,318	735	28.0	16.1	1.7

One fourth of the plants of these F_2 progenies were susceptible at 22° C., showing that the degree of resistance of the Wisconsin Hollander parent plants was not evident under these conditions. It is expected that some plants of the F_2 progenies from susceptible crosses and one fourth of the plants from resistant crosses will exhibit some resistance at lower soil temperatures (18° or 20°) or in the field. Field trials and tests at low soil temperatures with these progenies have not yet been carried out. The foregoing results, however, give further evidence that the resistance exhibited by Wisconsin Hollander is genetically different from and independent of that shown by the homozygous resistant lines. They further indicate that this resistance is a result of modifying factors.

DISCUSSION

The object of the investigation reported herein was to secure more exact information regarding the nature of resistance in Wisconsin Hollander to the yellows disease of cabbage. The evidence from this study and from observations of earlier workers indicates that with regard to resistance the Wisconsin Hollander variety consists of a heterogeneous group of individuals. This is shown by the F_1 progenies

from self-pollinated Wisconsin Hollander plants, which, when grown in the field, differed widely in their reaction to the disease, some being very susceptible, some highly resistant, and some of intermediate degrees of resistance.

The studies with the progenies from self-pollinated Wisconsin Hollander plants and from crosses with homozygous resistant and homozygous susceptible plants of other varieties indicate that the resistance displayed by Wisconsin Hollander is controlled by modifying factors. These factors of resistance are different in type and reaction from the single dominant factor controlling resistance in the homozygous resistant lines. The factors for resistance in Wisconsin Hollander are not dominant but are cumulative in effect. It is suggested that the two mother plants from which the Wisconsin Hollander variety was developed probably contained a considerable number of modifying factors. As a result of segregation through successive generations, plants of this variety would be heterogeneous in nature, differing from one another in the number and kind of the factors that produce resistance. Some of the plants, when grown under field conditions, would be very susceptible, some highly resistant, and many of intermediate degrees of resistance. This was shown to be true in field tests with progenies from self-pollinated Wisconsin Hollander plants (table 2), and partly accounts for the differences among commercial lots of Wisconsin Hollander. If propagation occurs for a period of years on soil free from the yellows organism, where elimination of susceptible plants is impossible, the percentage of susceptible plants may increase.

Genetical studies with Wisconsin Hollander $\times$ homozygous resistant and Wisconsin Hollander $\times$ homozygous susceptible crosses indicate that the modifying factors for resistance in Wisconsin Hollander are lacking in the two respective homozygous strains. The F_1 progenies from the susceptible crosses were very susceptible in the field tests. The relative degrees of resistance of the Wisconsin Hollander parents were partially expressed in these tests in that progenies from crosses with the more resistant Wisconsin Hollander parents showed a smaller percentage of diseased plants. These progenies were completely susceptible when grown in inoculated soil at a constant soil temperature of 22° C. The F_2 progenies from susceptible crosses were also very susceptible when grown in inoculated soil at a constant soil temperature of 22°. Assuming the absence of modifying factors for resistance in the homozygous susceptible plants, the F_1 progenies from crosses with Wisconsin Hollander would segregate for the factors of the Wisconsin Hollander parent, and therefore the degree of resistance would be decreased. The original resistance of the Wisconsin Hollander parents was not recovered in the F_2 generation. Total recovery of this resistance is expected in but few plants in successive generations if the number of factors is large. It is expected that some resistance will be shown by F_2 plants from the foregoing crosses if they are grown under lower soil-temperature conditions or in the field. Additional tests in this respect were impossible because of the limited seed supply. The F_2 progenies obtained by selfing plants of the resistant cross showed one fourth of the plants susceptible to yellows when grown in inoculated soil at 22° C. The only type of resistance obtained in these tests resulted from the

single dominant factor for resistance of the homozygous resistant parent.

Greater uniformity in resistance to the yellows disease was found in the individuals of F_1 progenies from self-pollinated Wisconsin Hollander plants than was found in Wisconsin Hollander plants in general. Segregation took place in these progenies, however, resulting in some individuals being more resistant and some less resistant than the mean. This was further shown in tests with F_2 progenies from self-pollinated Wisconsin Hollander plants (table 10), some of which were more resistant and others more susceptible than the corresponding F_1 progenies. There was also some difference in resistance among individuals of the F_2 progenies, showing that the character of resistance had not become stabilized by self-pollination in a period of two generations.

The resistance of Wisconsin Hollander as compared with that of homozygous resistant plants is of an intermediate type. The most resistant line from Wisconsin Hollander plants showed complete breakdown of resistance when grown in inoculated soil at a constant soil temperature of 24°C. , and but a small percentage of plants remained healthy at 22° ; whereas the plants of the homozygous resistant strains remained completely healthy in inoculated soil at temperatures as high as 26° . The resistance of Wisconsin Hollander can be increased by selection in self-pollinated lines, but whether a resistance equal to that of homozygous resistant lines can be developed in Wisconsin Hollander as a result of the accumulation of modifying factors remains to be determined by further study. Such a study should proceed by continued selection of the most resistant plants and self-pollination for two or more generations, until segregation ceases and a high degree of resistance becomes stabilized.

There appears to be a fine balance between the resistance of Wisconsin Hollander plants of the same genetical constitution and the aggressiveness of the pathogene in causing disease. By varying environmental conditions, such as soil temperature, this balance may be easily shifted to favor either the host or the pathogene. Such balances of slightly different planes are assumed for the F_1 progenies of slightly different degrees of resistance to yellows. The shifting of this balance in favor of the pathogene has been noted in various tests. Several F_1 progenies from self-pollination tested in the field in 1930 (table 2, class 3) contained very few diseased plants, and these showed but incipient symptoms. In the warmer summer of 1931, however, 50 percent or more of the plants of these progenies were diseased (table 3), and the diseased plants were more severely affected in general. This partial breakdown of resistance is even more evident on comparing the results of the two field tests of the F_1 progenies from susceptible crosses. Because of the higher degree of resistance found in strain 8-11s, the host-parasite relationship in this strain was but slightly affected by the increase in severity of weather conditions.

This relationship was also noticed in the greenhouse tests. There appears to be a quite definite limit within which the plants of a progeny are successful in resisting the fungus. This limit differs with the different progenies from self-pollinated Wisconsin Hollander plants, which showed various degrees of resistance. For example, very slight symptoms were found in a few plants of strain 8-11s at 16°C. ,

of strain 8-46s at 13° to 14°, and of strain 8-33s at 10° to 12°. At these temperatures the majority of plants remained healthy and plants showing symptoms were not retarded in growth but tended to recover. In each case, at slightly higher temperatures, the percentage of affected plants increased and the disease became more severe.

When plants were grown in inoculated soil at 22° C. the majority of those of the most resistant line from selected Wisconsin Hollander (8-11s) showed symptoms of the disease. Many such plants exhibited but slight symptoms and later recovered.

SUMMARY

This paper reports a study of the nature of resistance of the Wisconsin Hollander variety of cabbage to the yellows organism, *Fusarium conglutinans* Wr. A number of plants of this variety were self-pollinated and crossed with homozygous resistant and homozygous susceptible plants from other varieties. Selected plants from the F_1 progenies were self-pollinated.

Field and greenhouse trials were conducted in which the various progenies and commercial Wisconsin Hollander were tested for resistance to yellows in comparison with homozygous resistant and homozygous susceptible lines.

The Wisconsin Hollander variety contains a heterogeneous group of individuals. Under severe field conditions the individuals of F_1 progenies from self-pollinated Wisconsin Hollander plants ranged in degree of resistance from completely susceptible to highly resistant, indicating the existence of modifying factors for resistance. The original resistance of Wisconsin Hollander was not recovered in F_2 progenies from crosses with homozygous susceptible plants. In the F_2 progenies from the cross Wisconsin Hollander $\times$ homozygous resistant, when grown in inoculated soil at a constant soil temperature of 22° C., segregation resulted in 3 resistant to 1 susceptible.

Segregation of factors for resistance occurred in the F_1 and F_2 progenies from self-pollinated Wisconsin Hollander plants. Some F_2 progenies exhibited a higher degree of resistance and others a lower degree than did the progenies from which the mother plants were selected.

Individual plants of the more resistant progenies from Wisconsin Hollander are less severely affected by the disease than those of the susceptible progenies. Many plants of the former exhibit but slight symptoms in the field or at a controlled soil temperature of 20° to 22° C. The growth of such plants is not materially checked by the disease and they tend to recover.

The resistance of Wisconsin Hollander tends to be broken down when plants are grown in inoculated soil at constant soil temperatures ranging from 20° to 24° C. The most resistant line obtained from Wisconsin Hollander plants became 100 percent diseased at a constant soil temperature of 24°. In comparison with the resistance displayed by homozygous lines the resistance of Wisconsin Hollander is of an intermediate degree.

Wisconsin Hollander seedlings of various ages reacted quite similarly to the disease. There was no evidence of increased resistance

with age in seedlings within the limits studied. The rate of incidence of the disease was slower in seedlings grown from seed in inoculated soil than in 15-day-old plants transplanted to inoculated soil.

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INHERITANCE OF SEED COLOR IN CROSSES OF BROWN-SEEDED AND WHITE-SEEDED SORGHUMS¹

By JOHN B. SIEGLINGER

*Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry,
United States Department of Agriculture*

INTRODUCTION

In the improvement of sorghums by crossing, the problem of combining desirable characters of brown-seeded varieties with the white-seed character is of both practical and theoretical importance. Stock and poultry feeders generally recognize the greater palatability of white-seeded grain sorghums, and the market value of the better brown-seeded varieties is less than that of other grain sorghums.

Swanson² indicated the possible color factorial constitution of a number of brown-, white-, yellow-, and red-seeded sorghums. His conclusions regarding the color factors of the brown-seeded varieties, with the exception of Red Amber, which he determined by genetic experiments, were based upon a microscopic examination of the seed coats.

Data on inheritance of seed color in three crosses between brown-seeded and white-seeded varieties are presented in this paper.

CROSSES STUDIED

The crosses were made by the writer at the Southern Great Plains Field Station, Woodward, Okla., in 1928, 1929, and 1930. The F₂ or segregating generations were grown and observed in 1930, 1931, and 1932. The three crosses were (1) Scarborough broomcorn (C.I.³ no. 817) × a white-seeded fixed segregate of Freed sorgo × Evergreen Dwarf broomcorn, (2) Blackhull kafir (C.I. no. 813) × darso, and (3) Blackhull kafir (C.I. no. 813) × Sumac sorgo (F.C.I.⁴ no. 1712).

Cross no. 1 was made primarily to obtain a white-seeded variety of broomcorn. Scarborough broomcorn has the brown seeds typical of broomcorn. The other parent of this cross, a selection from a cross between Freed sorgo and Evergreen Dwarf broomcorn, was early in maturity and had white seeds and a broomcornlike head but with an inferior brush.

Cross no. 2 was made for the purpose of obtaining a white-seeded strain of darso. The kafir parent, known locally as Sharon or Santa Fé kafir, is white seeded and of medium early maturity. In common with other typical kafirs it has awnless glumes. Darso, the pollen parent, has reddish-brown seeds and is awned.

Cross no. 3 was made to obtain a white-seeded strain of Sumac sorgo. Sumac sorgo, the staminate parent, has very small, dark walnut-brown seeds. The other parent was the kafir used in cross no. 2. The feathery stigmas of the two parents differ in color, those of the kafir being white and those of Sumac sorgo a deep yellow.

¹ Received for publication July 3, 1933; issued November 1933.

² SWANSON, A. F. SEED-COAT STRUCTURE AND INHERITANCE OF SEED COLOR IN SORGHUMS. *Jour. Agr. Research* 37: 577-598, illus. 1928.

³ C.I. refers to accession number of the Division of Cereal Crops and Diseases.

⁴ F.C.I. refers to accession number of the Division of Forage Crops and Diseases.

The seed-color factors in these several crosses are designated ⁵ *B*, a factor for brown nucellar layer which also causes a brown in the epidermis; *b*, its allelomorph for absence of the brown nucellar layer; *R*, a dominant factor for red or yellow in the outer layers of the pericarp; and *r*, its allelomorph for lack of red or yellow in this region.

The seed colors of red kafirs and yellow milos appear different to the eye. The red and yellow seed characters have been found to differ from those of white seeds by single dominant factors. In a cross of Early Red kafir and Dwarf Yellow milo made in 1927 the *F*₂ population of 661 plants produced in 1929 did not contain any white-seeded plants. If the red and yellow color factors had been genetically distinct and independent, one sixteenth of the population, or about 41 plants, should have had white seeds. There were various shades of red, orange, and yellow, but these variations probably were due to genetic or physiologic modifiers, including differences in texture or thickness of the various layers of the pericarp and endosperm. This behavior indicates that the color in the seeds of red kafirs and yellow milos is due to the same single-color factor designated *R*.

EXPERIMENTAL RESULTS

SEED COLOR

In 1930 four 8-rod rows of the *F*₂ generation of the cross between Scarborough broomcorn and the white-seeded segregate of Freed sorgo × Evergreen Dwarf broomcorn were grown. Six 8-rod rows of the *F*₂ generation cross of Blackhull kafir × darso were grown in 1931. Three 8-rod rows of the *F*₂ generation cross of Blackhull kafir × Sumac sorgo were grown in 1932. Seed-color counts were made in each of these crosses. The data are shown in table 1.

TABLE 1.- Seed color in the *F*₂ generation of 3 crosses between brown- and white-seeded sorghums

Cross and items of comparison	Number of plants				χ^2	P
	Total	Nucellar layer present	Nucellar layer absent			
		Brown seeds	Red or yellow seeds	White seeds		
Scarborough broomcorn $\times$ white seeded segregate of Freed sorgo $\times$ Evergreen Dwarf broomcorn:						
Observed.....	733	552	144	37		
Calculated (12:3:1).....	733	549.8	137.4	45.8	2.017	0.366
Blackhull kafir $\times$ darso.						
Observed.....	1,367	1,020	269	78		
Calculated (12:3:1).....	1,367	1,025.3	256.3	85.4	1.30	.535
Blackhull kafir $\times$ Sumac sorgo						
Observed.....	684	557	141	36		
Calculated (12:3:1).....	684	513	128.3	42.7	2.38	.313

There was a segregation closely approximating a dihybrid 12:3:1 ratio for brown-, yellow-, and white-seeded plants in each of the three crosses.

⁵ SIEGLINGER, J. B. SEED-COLOR INHERITANCE IN CERTAIN GRAIN-SORGHUM CROSSES. Jour. Agr. Research 27: 53-64. 1924.

White-seeded segregates in the broomcorn cross were selected in F_2 , but the inheritance of seed color was not studied in later generations, except to note that the white segregates, when selfed, produced only white-seeded progeny, as expected. The F_3 generation of the Sumac sorgo cross has not yet been grown. The progeny of 50 selected F_2 heads of the Blackhull kafir $\times$ darso cross, of which 25 had been selfed, was grown in the F_3 generation in 1932. The breeding behavior of these strains is shown in table 2.

TABLE 2. —Breeding behavior in F_3 of the progenies from 50 selected F_2 heads of the Blackhull kafir $\times$ darso cross, 1932

Item	Number in F ₃ generation			Devi- ation from 3:1 ratio	Prob- able error of devi- ation	Suggested genotype
	Strains	Plants with -				
		Brown or buff seeds	Yellow seeds			
Brown seed	17					
Bred true - dark brown	3	All				<i>BBRR</i>
Bred true - buff	2	All				<i>BBrr</i>
Segregated - brown and buff	4	All				<i>BbRr</i>
Segregated - brown and yellow	1	184	53	6.25	4.50	<i>BbRR</i>
Segregated - brown (buff) and white	3	546		9.0	7.9	<i>Bbrr</i>
Segregated - brown, yellow, and white	4	729	190	(^a)		<i>BbRr</i>
Yellow seed	10					
Bred true - yellow	3		All			<i>bbRR</i>
Segregated - yellow and white	7		1,276	440	11	12.1
White seed	23					
Bred true - white	23			b All		<i>bbrr</i>

^a $P=0.37$ on basis of calculated 12.3:1 ratio

^b Except a few outcrosses from unbagged heads

The results obtained in the F_3 generation substantiate the 2-factor hypothesis indicated by the F_2 segregation in the three crosses. It seems evident that the brown-seeded varieties, Scarborough broomcorn, darso, and Sumac sorgo, have the factorial constitution *BBRR*, and that the white-seeded varieties have the double recessive factors *bbrr*. The F_1 brown-seeded plants have the factors *BbRr*. The nine possible genotypes, all of which were demonstrated by the F_3 generation behavior of the Blackhull kafir $\times$ darso cross, are shown in table 2.

AWNS

A study of the inheritance of awns was made in the Blackhull kafir $\times$ darso cross. The kafir parent is awnless, while darso has well-developed straight awns averaging about one half inch in length. The F_1 glumes were awnless like those of the kafir parent, but in the F_2 generation there was a segregation into awned and awnless types. The segregations for awn type and seed color are shown in table 3.

A total of 1,052 plants were awnless and 315 were awned, a deviation of 26.75 ± 10.79 from a calculated 3:1 ratio that probably is not significant. The data in table 3 show a good fit for independent segregation of the factors for awn type and seed color and indicate an absence of linkage.

TABLE 3.—*Awn type and seed color, in the F₂ generation, of the 1,367 plants of the Blackhull kafir × darso cross, 1931*

Awn type and seed color	Number of plants		Awn type and seed color	Number of plants	
	Observed	Calculated (36 12 9 3 3 1)		Observed	Calculated (36 12 9 3 3 1)
Awnless, brown	788	768.9	Awnless, white	58	64.1
Awned, brown	232	256.3	Awned white	20	21.4
Awnless, yellow	206	192.2			
Awned, yellow	63	64.1	Total	1,367	(*)

* $\chi^2=4.46$; $P=0.488$.

In the F₃ generation (table 4) all awned plants were obtained from the 14 awned F₂ heads. The 36 awnless heads produced only 5 progenies that were all awnless; the remaining 31 progenies were heterozygous for awns and segregated into a total of 5,962 awnless and 1,800 awned plants, a deviation of 140.5 ± 25.73 from a 3:1 ratio. With random sampling, 36 awnless F₂ heads should produce 12 all-awnless rows, whereas only 5 were produced. The heads used for seed were selected for size of head and seed, small glumes, and the shape of head approximating that of darso. This selection may account for the excess of heterozygous over homozygous awnless heads. The considerable excess of awnless heads in the segregating progenies may have resulted partly from natural crossing in the open-pollinated heads. The segregation in the selfed F₃ lines (table 4) as well as in the F₂ generation (table 3) was in close agreement with the expected numbers from a simple 3:1 ratio.

TABLE 4.—*Awnedness in F₃ of the progenies from 50 selected F₂ heads of the Blackhull kafir × darso cross in 1932*

Awn character in F ₂ and breeding behavior in F ₃	Number of strains		Number of F ₃ plants			Probable error of deviation
	Total	Selfed	Awnless	Awned	Deviation from 3:1 ratio	
Awnless						
Bred true—awnless	5	2	All			
Segregated (selfed lines)	14	14	2,615	848	17.75	17.19
Segregated (open-pollinated)	17	0	3,347	952	122.75	19.20
Total	31	14	5,962	1,800	140.5	25.73
Awned						
Bred true—awned	14	9		(*)		

* Except a few outcrosses from unbagged heads.

STIGMA COLOR

To study the relation, if any, between stigma color and seed color, the plants of the F₂ generation of the Blackhull kafir × Sumac sorgho cross were tagged according to stigma color during the blooming period. Later, in classifying the seed color, the stigma color was noted from the tags. The following results were obtained:

All the 36 white-seeded plants had white stigmas.

All the 141 yellow-seeded plants had yellow stigmas.

One hundred and ten of the brown-seeded plants had white stigmas.

Three hundred and ninety-seven of the brown-seeded plants had yellow stigmas.

The brown seeds from flowers with white stigmas were lighter in color than those from flowers with yellow stigmas and could best be described as tan or buff like the varieties and segregates having the *Br* factors. Judged from the stigma color of the white- and yellow-seeded segregates, it may be concluded that in this cross the yellow stigma color is merely another expression of the presence of the *R* color factor and that the white stigma indicates an absence of the *R* factor, or that complete linkage existed between the *R* factor and the colored stigma. This knowledge should make it possible to identify one fourth of the white-seeded types at the beginning of the blooming period and thus eliminate the bagging in the three fourths of an F_2 population having the *R* factor for seed color when white-seeded segregates only are desired.

SUMMARY

Data on the inheritance of the seed color of three crosses between brown-seeded and white-seeded sorghums are presented.

The brown seed color of Scarborough broomcorn, darso, and Sumac sorgo is the result of two seed-color factors, *B* and *R*.

In the F_2 generation of crosses between the brown-seeded and white-seeded sorghums, a ratio of 12 brown- to 3 yellow- to 1 white-seeded plant was obtained.

The awnless condition in Blackhull kafir is dominant to the presence of awns in darso, and a segregation into 3 awnless to 1 awned was observed in the F_2 generation of this cross. There was no apparent linkage of the seed color and awn factors.

The stigma color and its relation to seed color was studied in the F_2 generation cross of Blackhull kafir $\times$ Sumac sorgo. Yellow stigma was completely associated with the presence of the *R* seed-color factor.

SUBERIZATION AND WOUND-CORK FORMATION IN THE SUGAR BEET AS AFFECTED BY TEMPERATURE AND RELATIVE HUMIDITY¹

By ERNST ARTSCHWAGER, *pathologist*, and RUTH COLVIN STARRETT, *assistant cytologist*, *Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

A large percentage of sugar beets are stored at harvest before passing through the factory, and, according to Tompkins and Nuckols,² in such storage piles crown rot frequently develops. These writers found the resulting injuries most pronounced in beets topped at or below the line of the lowest leaf scar and only slight where in topping a large part of the crown was allowed to remain. Unable to account for this differential decay, they suggest that a study of wound-cork formation might aid in the solution of the problem.

While plant organs, such as tubers, fleshy roots, and corms, normally respond to wounding by rapid suberization of the cut surface and a subsequent development of a firm cork layer, Priestley and Swingle³ have found that beets form a visible suberin deposit only slowly.

The studies reported in this paper were initiated to determine the influence of temperature and humidity on the rate of suberization and wound-cork formation on beets topped at different planes, the method of procedure being essentially the same as that employed in the study of wound-periderm development in sweetpotato and gladiolus.⁴

STRUCTURE OF THE MATURE BEET

The sugar beet (fig. 1) is an elongated, more or less pear-shaped body composed morphologically of crown, neck, and root. In cross section it shows a small, star-shaped core surrounded by a number of annular zones or rings of growth. Each annular zone is made up of a concentric ring of vascular tissue and storage parenchyma. The vascular tissue is composed of numerous collateral bundles which anastomose tangentially, forming a network of connecting bundles. In the narrow tapering zone of the beet the rings unite with one another gradually, but a more abrupt fusion is effected in the crown. This zone is composed essentially of intricately anastomosing vascular bundles mostly devoid of the parenchymatous elements so characteristic of the main body of the beet. In the upper region of the neck the central core opens up and encloses pith, which, as the neck passes into the crown, widens and frequently becomes hollow.

¹ Received for publication June 2, 1933; issued November 1933.

² TOMPKINS, C. M., and NUCKOLS, S. B. THE RELATION OF TYPE OF TOPPING TO STORAGE LOSSES IN SUGAR BEETS. *Phytopathology* 20: 621-635, illus. 1930

³ PRIESTLEY, J. H., and SWINGLE, C. F. VEGETATIVE PROPAGATION FROM THE STANDPOINT OF PLANT ANATOMY. U.S. Dept. Agr. Tech. Bul. 151, 99 p., illus. 1929

⁴ ARTSCHWAGER, E., and STARRETT, R. C. SUBERIZATION AND WOUND-PERIDERM FORMATION IN SWEETPOTATO AND GLADIOLUS AS AFFECTED BY TEMPERATURE AND RELATIVE HUMIDITY. *Jour. Agr. Research* 43: 353-364, illus. 1931.



FIGURE 1.—Sugar beet cut to show transverse and tangential surfaces.

SUBERIZATION AND WOUND-CORK FORMATION

The rate of suberization and wound-cork formation as determined from a series of samples taken from an experiment in which temperatures and humidities were closely controlled is given in figure 2, the lightly shaded area indicating surface suberization and the heavily shaded area deeper suberin deposits. The latter, however, even under optimum conditions is slow in forming, at best involving only one or two cell layers. The contour of the suberized zone appears to be related to the tissues exposed in the cut. In the narrow, cortical region the suberin layer is sunk more or less deeply into the tissue beneath the exposed surface, while in other regions the topography of this layer is practically flat.

At low temperatures suberization of the cut surface is both slow and shallow; at higher temperatures its development is initiated after the third day and maximum development is attained after 5 to 7 days. Changes in relative humidity seem to have little influence on surface suberization, except that at maximum storage temperature the humidities below saturation seem to stimulate an early development of deeper-reaching suberin deposits. This condition is contrary to observations made on sweetpotato and gladiolus, which reacted favorably to increases in the relative humidity.

Since suberization precedes wound-periderm formation, the factors governing the former process will also affect the latter. However, while somewhat lowered humidities expedited suberization, a wound periderm would only develop when both temperature and relative humidity were high. The periderm layer, however, was very shallow (fig. 3), even under optimum conditions for its development. The first periderm cells were observed in the cortex of the cut surface, and this layer also attained its maximum thickness at this point. This ready response of cortical cells to wounding is even more pronounced in sweetpotato and gladiolus than in beet, and very likely it is a general phenomenon.

A comparative study of wound healing in crown, neck, and tap region of beets showed little difference in either manner or rapidity of suberization and wound-cork formation. In beets topped through the upper crown suberization was somewhat more rapid, but a wound periderm was slow in forming and could not have been a factor in preventing early infection in storage piles. The fact remains, however, that crown topping results in a considerable decrease in the percentage of decay in beets in storage piles as compared with that in beets topped at or below the lowest leaf scar; and since difference in suberization rate in beets cut through the crown or below the leaf scars is rather slight, the peculiar anatomical structure of the crown may in itself constitute an effective barrier to pathogenes. Here the vascular bundles anastomose freely and older bundles run obliquely, almost horizontally from the core to the periphery. There is little intervening parenchyma but a great deal of phloem tissue, and because the bundles run mostly horizontally there are also fewer exposed bundle ends. Phloem is known to actively stimulate suberin deposits, and its preponderance in the crown region may possibly cause the early formation of a slight yet effective suberin deposit, although the latter may not be detectable with the common reagents.

In the selection of mother beets for seed production, borings are commonly made for the removal of pulp for sugar analysis (fig. 4).

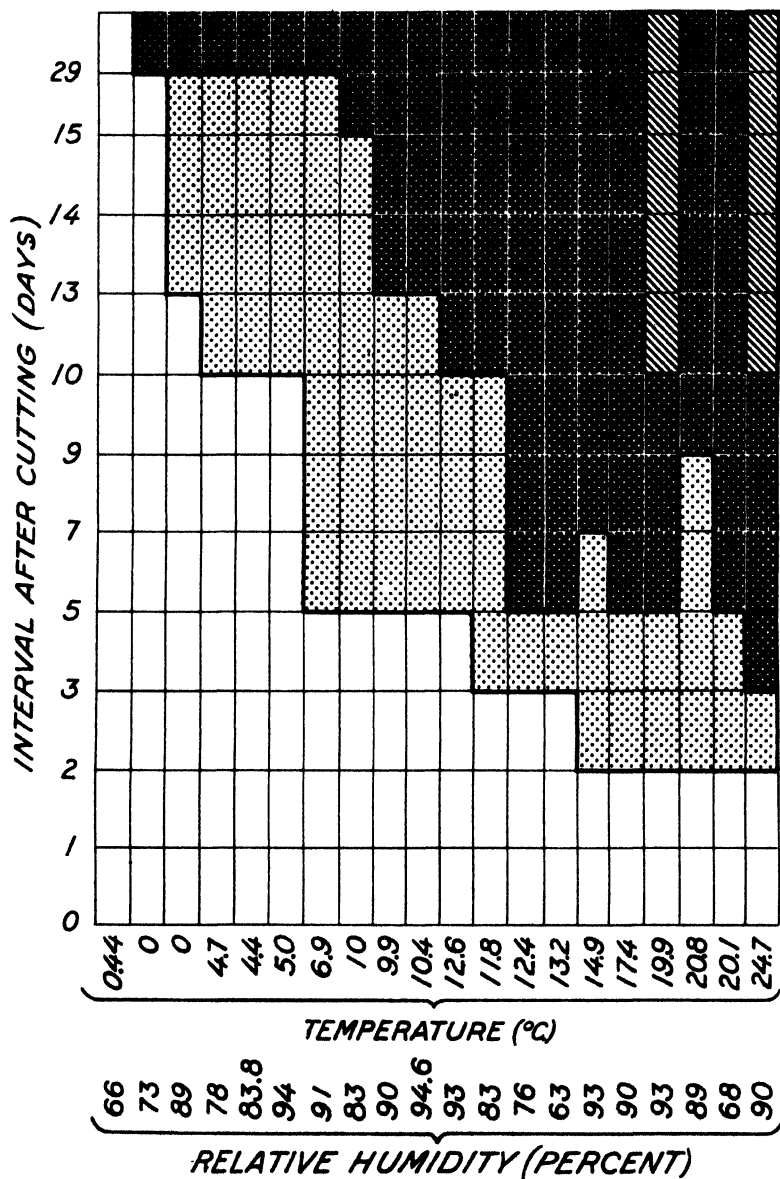


FIGURE 2.—Suberization and wound-periderm formation in sugar beet. Light shading indicates surface suberization; heavy shading, deeper suberin deposits; and hatching, wound periderm.

The outer surface of such a bore from beets that had been kept at a low storage temperature for a number of months was dark and when cut with a knife proved to be hard and gritty. Its outer zone, several cells deep, was lignified, and only one cell layer between lignified and

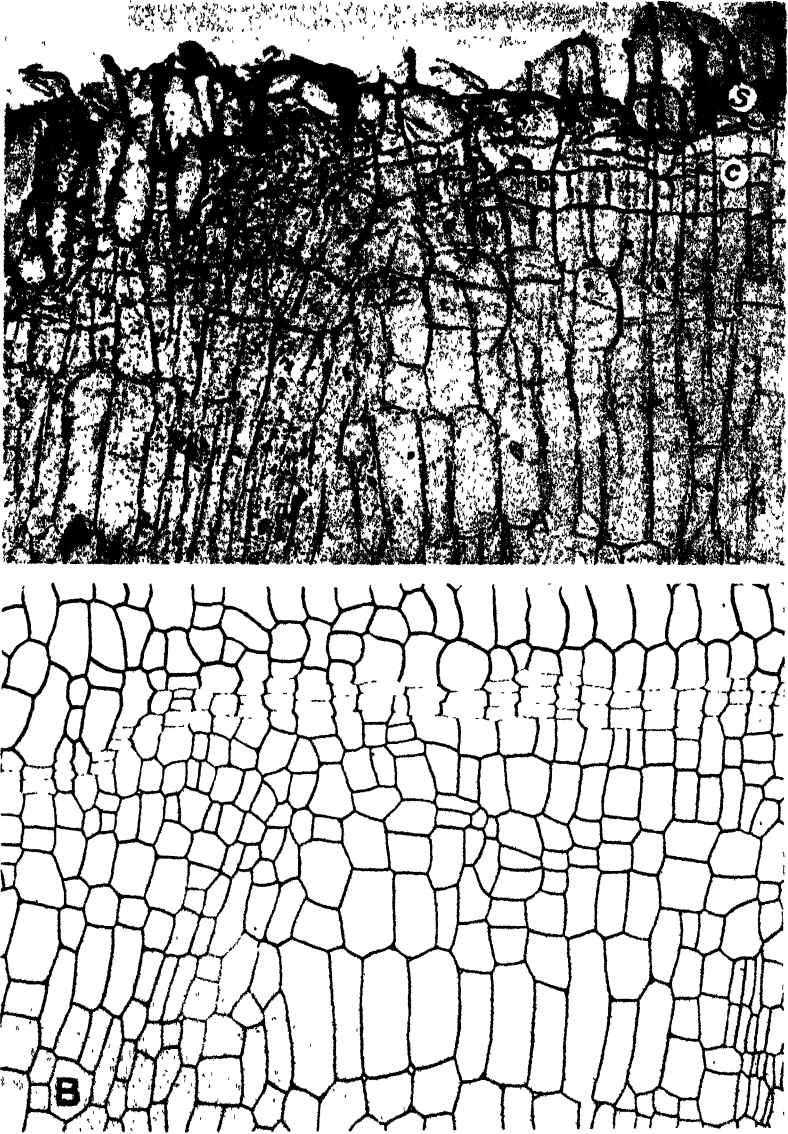


FIGURE 3.--A, Wound-periderm formation in sugar beet kept for 10 days at a temperature of 19.9° C. and a relative humidity of 93 percent; s, suberized; c, wound cork; B, detailed drawing of A.

normal tissue took the purple ammoniacal gentian-violet stain. There was local evidence of a wound periderm, but this tissue never formed an uninterrupted concentric band, and some material showed no

evidence whatsoever of wound-periderm formation. Although no observations were made on the time factor in suberization, it was evident that the lignified and suberized zone bordering the bore hole afforded considerable protection against infection.

The much-belated appearance of wound cork in this material indicates that even at low storage temperatures a periderm may eventually develop, although no periderm formation was apparent in the course of an experiment that ran for 30 days. This observation on mother beets is comparable with the results obtained with gladiolus corms, in which at low temperature a periderm developed only after 79 days.⁵

SUMMARY

The roots of sugar beet respond to wounding by suberization of the cut surface and a belated development of a shallow wound periderm. Suberization is very slight; it involves, at best, only one or two layers of cells, and if temperature and relative humidity are not favorable it is found only in the outer wall. Under favorable conditions, a wound periderm develops as early as 10 days after wounding, but this layer is very shallow. There is little difference in the healing of wounds in beets cut through the crown, neck, or tap region, and the relative scarcity of decay in beets topped through the crown is probably bound

FIGURE 4.—Longitudinal section of sugar beet with bore hole.

up with the specialized anatomical structure of this region. The tissues bordering bore holes made through beets become lignified and slightly suberized. In addition there is slight localized periderm formation.

⁵ ABTSCHWAGER, E., and STARRETT, R. C. (See footnote 4.)

LEPTOSPHERIA SALVINII, THE ASCIGEROUS STAGE OF HELMINTHOSPORIUM SIGMOIDEUM AND SCLEROTIUM ORYZAE¹

By E. C. TULLIS

Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Stem rot of rice (*Oryza sativa* L.), caused by *Sclerotium oryzae* Catt., has long been known to cause severe losses to rice growers both in the United States and in foreign countries. The fungus was first described from Italy in 1876 by Cattaneo (3).² In 1907 Metcalf (8) reported that it had been found in North Carolina and that it "caused a stem rot under water."

In 1921 Tisdale (18) reported the occurrence of the disease in Louisiana and stated that he suspected its occurrence in Arkansas. He also demonstrated the pathogenicity of the fungus by adequate inoculation experiments. In 1926 Young (22) definitely reported the disease in Arkansas.

In 1910 Miyake (9) reported the occurrence of stem rot in Japan. He stated that great losses were caused by this disease because of the failure of the rice grains to fill properly. Subsequent investigations were made by Sakurai (14), Hemmi and Yokogi (7), and others. In Cochinchina the disease was reported by Vincens (21) in 1923. In 1913 Shaw (15) and Butler (2) stated that stem rot occurred in India and that one of the most conspicuous symptoms was the stimulation of tillering, a symptom that has not been observed in the United States. Additional reports of stem rot have been made from various parts of India by Bryce (1), Thompson and Sawyer (17), Rhind (13), and Park (10); from the Philippines, by Teodoro and Bogayong (16) and Reyes (11, 12); and from Bulgaria by Dodoff and Kovachevsky (6).

All the investigators mentioned except Vincens give only the sterile fungus, *Sclerotium oryzae*, as the cause of stem rot. Vincens (21), on his return to France from Cochinchina, carried on a series of experiments with *S. oryzae* and reported the development of a number of conidial forms associated with it in culture. These were *Verticillium* sp., *Beauveria oryzae* Vincens, forms alpha and beta; *Fusarium oryzae* Vincens, forms alpha and beta; and *Acremonium fuliginosum* Vincens. Descriptions and illustrations of these were given, but it was not shown that any of them were connected with the stem-rot fungus, *S. oryzae*.

The writer (19, 20) has previously reported briefly the results of investigations showing that the stem-rot fungus (*Sclerotium oryzae* Catt.), has a conidial stage (*Helmintosporium sigmoideum* Cav.)

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² Reference is made by number (italic) to Literature Cited, p. 685.

and also an ascigerous stage (*Leptosphaeria salvinii* Catt.). The present paper reports these results in full.

HELMINTHOSPORIUM SIGMOIDEUM, THE CONIDIAL STAGE OF SCLEROTIUM ORYZAE

In the summer of 1930 the writer found conidia and conidiophores of a *Helminthosporium* on agar cultures of *Sclerotium oryzae* and also on some seedlings of rice grown under aseptic conditions in test tubes and inoculated with a pure culture of *S. oryzae*. Inoculations with sclerotia of *S. oryzae* on other sterile rice seedlings gave the same results. Transfers made at the same time from the original culture failed to show the presence of any contaminating organism. More extensive inoculations and a detailed study of the conidial stage were then made. In the fall of 1931, 16 commercial varieties of rice grown at the Rice Branch Experiment Station, Stuttgart, Ark., were examined for this *Helminthosporium*. Spores and conidiophores of the fungus were found on mature stubble of all the varieties.

SOURCE OF CONIDIA

Sterile rice seedlings grown on agar in test tubes were inoculated with cultures of *Sclerotium oryzae* from the United States, Japan, and India, respectively. In these experiments eight cultures of *S. oryzae* from the following sources were used:

Culture no. 1 isolated by V. H. Young, Department of Plant Pathology, University of Arkansas, from diseased rice collected by him in the vicinity of Stuttgart, Ark.

Culture no. 2 isolated by the writer from diseased rice collected by him in the vicinity of Beaumont, Tex.

Culture no. 3 received from the Centraal Bureau voor Schimmellecultures, Baarn, Netherlands. This culture was isolated from diseased rice in Japan by K. Nakata, Kyushu Imperial University, Fukuoka, Kyushu, Japan.

Culture no. 4 obtained from India through the courtesy of M. Mitra, imperial mycologist, Imperial Institute of Agricultural Research, Pusa, India.

Cultures nos. 5 to 8 obtained from India through the courtesy of B. B. Mundkur, of the Imperial Institute of Agricultural Research, Pusa, India.

In all the inoculations in test tubes the sclerotia were placed on the agar at the side of the seed after the seed had germinated and the seedling had been found to be sterile. Following inoculation, the seedlings were incubated at about 25° C. The seedlings were not wounded.

On January 7, 1930, culture no. 1 was used in making inoculations on five sterile Blue Rose rice seedlings grown singly in test tubes on corn-meal agar. Fifteen days later conidia of a *Helminthosporium* had been produced on four of the seedlings. By that time all the inoculated seedlings had succumbed to the attacks of the fungus, and typical sclerotia were found in them. The check seedlings in the uninoculated test tubes remained alive and sterile.

On February 10 culture no. 2 was used in making similar inoculations on 26 sterile seedlings of Blue Rose rice. All the seedlings were killed by the fungus and at the end of 3 weeks conidia of a *Helminthosporium* were noted on 20 of them. Sclerotia were produced in all cases. Five uninoculated check plants remained alive and sterile.

Culture no. 3 was used on February 10 in similar inoculations on seven sterile Blue Rose rice seedlings. The *Helminthosporium* conidial stage was subsequently found on two of them. Six of the seed-

lings were killed by the fungus. Four uninoculated check plants remained alive and sterile.

On June 7, 1930, culture no. 4 was used in similar inoculations on six sterile seedlings of Fortuna rice. The inoculated seedlings were killed by the fungus but no spores were produced on any plant. Characteristic sclerotia, however, were produced in one plant and in the agar of that tube. The uninoculated check plants remained alive and sterile.

No inoculation of seedlings was made with cultures 5, 6, 7, and 8. However, cultures 5, 6, and 7 produced the typical conidial stage when transferred to corn-meal agar slants with a 4 percent agar base.

The results of these inoculations indicated that under the conditions of the experiments the strains of the stem-rot fungus from the United States, Japan, and India are similar in their ability to produce conidia of a *Helminthosporium*.

CULTURAL STUDIES

Cultural studies were made of the *Helminthosporium* obtained from the inoculations of sterile rice seedlings just described. Corn-meal-agar dilution plates were prepared with the conidia to determine whether they were viable and to study the type of germination.

On February 10, 1930, four plates were poured from the conidia produced from culture no. 2. Germination was noted 12 hours later. Germ tubes had been produced from one or both end cells of the conidia and an arborescent mycelium was developing. Forty-eight hours later the growth was visible to the naked eye. At the end of a week small white spherical bodies had developed in all the cultures. These finally matured and proved to be the typical sclerotia of *Sclerotium oryzae*, which, when transferred to corn-meal-agar slants, produced typical colonies and sclerotia. The plates were kept under observation for 1 month, but no conidia were produced in any of the cultures.

On August 1, 1930, a second set of four dilution plates was prepared with conidia from transfers of culture no. 3 made June 15, 1930. When growth was visible, 48 hours after the plates were poured, 12 single spores were located with the aid of a microscope. These were then transferred to corn-meal-agar slants. At the end of 2 weeks each of these cultures had produced sclerotia typical of *Sclerotium oryzae*. For more than 3 months these cultures were examined frequently for conidia, but none was found in any of the 12 cultures.

On December 7, 1930, another set of dilution plates was poured with conidia from the transfers of culture no. 3. These conidia were slow in germinating. On the third day, however, germination began; some of the conidia did not germinate until the fourth day. Typical growths were produced. At the end of the first week there appeared in the agar irregular dark sclerotoid bodies with coarse, rather short, dark-colored radiating hyphae. Some of the bodies were completely embedded; others were formed at the surface of the agar. The submerged bodies (fig. 1, A) produced conidia sparsely (fig. 1, B); those on the surface produced conidia abundantly (fig. 1, C).

Soon after the discovery of the conidia all the cultures of *Sclerotium oryzae* in the laboratory were examined. Of the 30 available tubes of cultures 1, 2, 3, and 4, *Helminthosporium* conidia were found in 9; these represented cultures 1, 2, and 3 only. Later, conidia were found also in no. 4, as well as in nos. 5 to 8.

Another set of dilution plates was poured with *Helminthosporium* conidia produced on the rice seedlings that had been inoculated in test tubes with culture no. 2, as previously described. From these

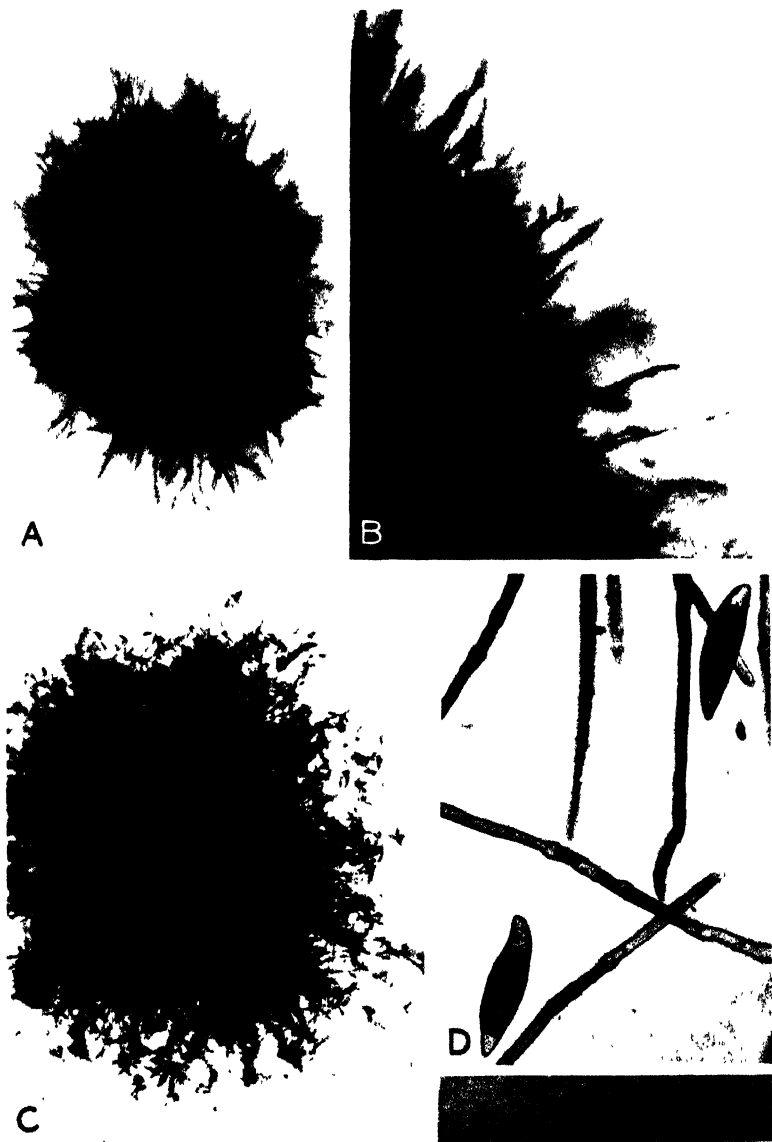


FIGURE 1.—A, Submerged sclerotoid body. $\times 25$. B, Portion of submerged sclerotoid body showing sparse sporulation. $\times 60$. C, Sclerotoid body produced on surface of agar, showing abundant conidia of *Helminthosporium sigmoideum*. $\times 25$. D, Conidia and portions of conidiophores of *H. sigmoideum*. $\times 480$.

plates 15 single-spore colonies were transferred to separate tubes of corn-meal agar. In each of the tubes sclerotia were produced but no conidia were found in any of them. Sclerotia were transferred from

these tubes to other tubes of corn-meal agar. Each of these produced sclerotia again and 11 of them also produced typical conidia of *Helminthosporium sigmoideum*.

All the cultures of *Sclerotium oryzae* produced typical spores of *Helminthosporium sigmoideum*.

INOCULATION EXPERIMENTS

With *Helminthosporium* conidia from rice seedlings that had been grown in test tubes and inoculated with *Sclerotium oryzae*, inoculations were made on individual leaves of sterile Blue Rose rice seedlings growing singly in test tubes on corn-meal agar. One leaf of each seedling was inoculated by placing on it, by means of a transfer needle loop, a small drop of sterile water in which the conidia were in suspension. Following inoculation, the seedlings were kept in diffuse light in the laboratory. After 3 days the inoculated leaves of two plants showed infection in the form of small brown elongated spots. The further development of the fungus from these spots killed the seedlings in 2 weeks. Typical *Helminthosporium* conidia were produced on the seedlings and sclerotia typical of *S. oryzae* were formed in the crown tissues of the seedlings and on the agar.

On March 21, 1930, leaves of 21 Blue Rose rice seedlings growing in soil in the laboratory were inoculated by placing a loop of a conidial suspension on each leaf. These plants were incubated for 24 hours in a saturated atmosphere at room temperature. Three days later brown lesions were observed on the leaves of 17 of the inoculated seedlings. In tissue platings made from these seedlings *Sclerotium oryzae* was obtained in only one, in which characteristic sclerotia and conidia developed. Later, microscopic examination was made of the remaining seedlings after they had been killed by the fungus, and on one additional plant typical conidia of *Helminthosporium sigmoideum* were found on sheaths just above the crown.

IDENTIFICATION

A study of the conidial stage showed that the fungus corresponded in all essential details with *Helminthosporium sigmoideum*, described and illustrated by Cavara (5, pl. 1, fig. 5) in 1889. Cavara's original description (5, p. 185) follows:

Effusum, atrum; hyphis fertilibus sparsis erectis, rigidiusculis, hinc inde nodulosis 8-10-septatis, simplicibus, olivaceis, 100-150 $\times$ 5 μ ; gonidiis magnis, falcato-sigmoideis, utrinque obtusis, triseptatis, cellulis mediis crassioribus, granulosis, dilute olivaceis, extimis hyalinis 55-65 $\times$ 11-14 μ .

Sur les graines, les feuilles, les tiges de l'*Oryza sativa*. Environs de Pavie. Été et automne. Forme voisine du *H. hyalophloeum* Sacc. (Fung. Ital. 814). Qui a cependant des hyphes fasciculées et des gonidies plus petites et point sigmoïdes de forme.

Cavara's illustrations are here reproduced as figure 2. Cavara (5) reported also the occurrence of *Sclerotium oryzae* from the vicinity of Pavie and referred to a specimen distributed in exsiccati.³ The writer examined this specimen, in the mycological collections of the Bureau of Plant Industry, United States Department of Agriculture, and found conidia of *Helminthosporium sigmoideum* as well as sclerotia of *S. oryzae*. Portions of conidiophores, also found in this specimen,

³ BRIOSI, G., and CAVARA, F. I. FUNGHI PARASSITE DELLE PIANTI COLTIVATE OD UTILI ESSICCATI, DELINEATI E DESCRITTI. Fasc. I, no. 25. 1888.

bore the sharp-pointed sterigmata characteristic of the conidiophores as noted by the writer (fig. 3). The conidia and conidiophores from the Briosi and Cavara specimen were found to be essentially identical with those from the writer's material.

From the cultural studies and artificial inoculations here recorded it is evident that *Helminthosporium sigmoideum* and *Sclerotium oryzae* are the conidial and sclerotial stages, respectively, of the same fungus.

LEPTOSPHAERIA SALVINII, THE ASCIGEROUS STAGE OF SCLEROTIUM ORYZAE

On some plants of Blue Rose rice and red rice grown in the greenhouse at Fayetteville, Ark., and infected with *Sclerotium oryzae*, a few peritheciump-like fruiting bodies were found on the outer sheaths. These fruiting bodies contained spores that were similar to the conidia of *Helminthosporium sigmoideum*.

Further examination of additional specimens revealed other such fruiting bodies with the spores in asci. These asci were found in various stages of development. The fungus was identified as *Leptosphaeria salvinii*, which had been described on rice in Italy by Cattaneo (4) in 1879. In 1910, Miyake (9) reported that this fungus had not been found in Japan.

In October 1931, perithecia of *Leptosphaeria salvinii* were found on a number of rice varieties infected with *Sclerotium oryzae* at the Rice Branch Experiment Station, Stuttgart, Ark. On these perithecia, conidiophores and conidia similar to those of *Helminthosporium sigmoideum* were frequently found. This, together with the similarity of the ascospores and conidia and the fact that the perithecia were

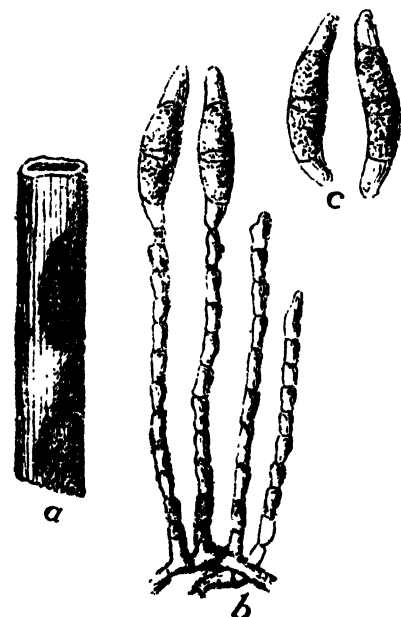


FIGURE 2.—Reproduction of Cavara's figures (5, pl. 1, fig. 5), the legend for which may be translated as follows: "*Helminthosporium sigmoideum* n. sp.: a, portion of stem of *Oryza* with spots caused by the fungus; b, fruiting hyphae; c, spores."

found on plants infected with *Sclerotium oryzae*, suggested the possibility that *L. salvinii* might be the ascigerous stage of *H. sigmoideum* and *S. oryzae*, which already had been shown to be connected. With this in mind, cultural studies and inoculation experiments were undertaken.

CULTURAL STUDIES

On November 17, 1931, three perithecia of *Leptosphaeria salvinii* from Blue Rose rice plants grown in the greenhouse at Fayetteville, Ark., were broken up in separate drops of distilled water to which dilute lactic acid had been added. The spore suspensions were smeared over the surface of slants of tubes of potato-dextrose agar. On the second and third days after the inoculations, 36 single-spore

colonies were located with the aid of a microscope and marked. Each colony was transferred to a separate tube of potato-dextrose agar (series 1).

On November 20 three additional perithecia were similarly broken up in distilled water to which a trace of lactic acid had been added. The drops of water were placed in sterile Petri dishes and diluted with cooled melted agar. On the second day thereafter nine transfers of single-spore colonies (series 2A) and three transfers from mycelium produced from two or more ascospores (series 2B) were made, as in the preceding experiment.

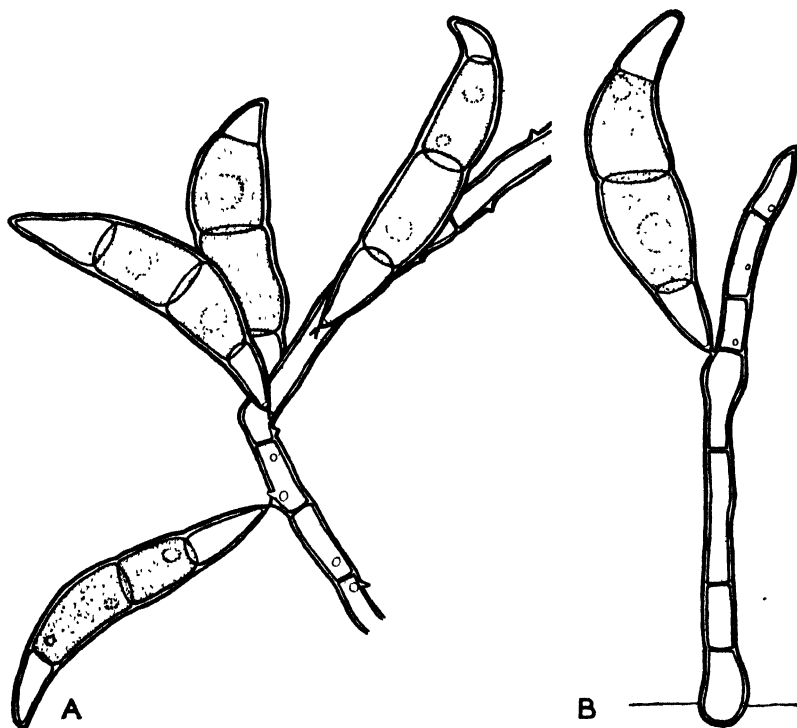


FIGURE 3 - *Helminthosporium sigmoideum*, $\times 1,140$. A, Conidia and a portion of conidiophore from a test-tube culture on corn-meal agar B, conidium and conidiophore from inoculated Blue Rose rice seedling grown in soil in the laboratory

On December 4 dilution agar plates were prepared, as previously described, from perithecia from Blue Rose rice grown in the greenhouse. Three single-spore cultures were secured (series 3).

On December 9, 1931, dilution agar plates were prepared of spores from perithecia produced on red rice in the greenhouse. Three single-spore cultures were secured from these plates also (series 4).

By the last of January 1932 all of the 54 cultures of *Leptosphaeria salvinii* (series 1 to 4) had produced abundant mycelium and sclerotia typical of *Sclerotium oryzae*. On January 23, 1932, one culture from series 2A and one from series 2B produced conidia typical of *Helminthosporium sigmoideum*. By the last of February 1932, 15 of the colonies from all four series of isolations had produced conidia typical of *H. sigmoideum*.

Conidia from isolation series 1 and 2 were transferred on February 26, 1932, to corn-meal-agar slants. They had germinated by the following day, and nine single-spore colonies were located with the aid of a microscope and transferred to individual tubes. By the middle of April, five of the nine cultures had produced sclerotia typical of *Sclerotium oryzae*. No sclerotia were produced by the other four cultures. No conidia were produced in any of the nine cultures during the period that they were under observation.

Ascospores of *Leptosphaeria salvinii* from rice stubble collected at Stuttgart, Ark., intermittently from October through February germinated readily when placed in tap water at room temperature. Some of the ascospores of the October collection germinated readily the following May in distilled water at room temperature.

The characteristics of the cultures from ascospores were essentially identical with those from either conidia or sclerotia.

Numerous unsuccessful attempts have been made to produce the ascigerous stage in artificial culture, by using cultures originating from each of the three stages.

INOCULATION EXPERIMENTS

Blue Rose rice plants were grown in autoclaved virgin soil from the vicinity of Stuttgart, Ark., in three 3-gallon stoneware jars. The seeds were prepared for sowing by removing the hulls, surface-sterilizing them for 5 minutes in 1 : 1,000 mercuric chloride solution, and then rinsing them in tap water. About 10 plants were grown in each jar.

On June 21, 1932, when the plants were 6 to 8 inches high, they were inoculated with sclerotia from pure cultures originating from ascospores of *Leptosphaeria salvinii*. These cultures were obtained from stem-rotted Blue Rose and Rexoro rice plants grown at Stuttgart in 1931. No plants were left uninoculated as controls, because in numerous trials plants grown similarly without inoculation had always remained free from stem rot. Inoculation was made by placing sclerotia in contact with the plants at the water line without wounding. On August 8, 1932, all culms that had not become infected as a result of the first inoculation were reinoculated with sclerotia from the same sources as were those in the first inoculation. When the reinoculations were made the outer sheaths of a number of the plants had been killed by the stem-rot fungus as a result of the first inoculation, and the typical symptoms had been produced. Examination of the dead sheaths a week later showed mature perithecia of *L. salvinii*. In addition, large numbers of conidiophores and conidia of *Helminthosporium sigmoideum* were found on the outer sheath surfaces, and sclerotia typical of *Sclerotium oryzae* were found embedded in the tissues of the sheaths.

It is evident from the results here set forth that *Leptosphaeria salvinii* is the ascigerous stage of *Helminthosporium sigmoideum* and *Sclerotium oryzae*.

TECHNICAL DESCRIPTION OF LEPTOSPHAERIA SALVINII CATT., 1879 (4, p. 126-127; pl. XV, figs. 1-3)

Synonym for sclerotial stage, *Sclerotium oryzae* Catt., 1876 (3); synonym for conidial stage, *Helminthosporium sigmoideum* Cav., 1889 (5).

MYCELIUM

Hyphae white to olivaceous, septate, profusely branched, 2μ to 5μ in diameter. In culture, mycelium white at first, later smoky to black at surface of medium. On host, mycelium white inside culm, olivaceous outside. Numerous irregular olivaceous appressoria form on culm; range from 14μ to 30μ by 8μ to 24μ .

SCLEROTIAL STAGE

Sclerotia spherical or nearly so, black at maturity, surface nearly smooth, at times covered with cottony web of white mycelium; 180μ to 280μ , mostly 230μ to 270μ . Habitat, in sheaths and culms of *Oryza sativa* (fig. 4) and *Zizaniopsis miliacea* (Michx.) Doell and Asch.

CONIDIAL STAGE

Conidiophores dark colored, septate, erect, simple or sparsely branched, 4μ to 5μ by 100μ to 175μ ; conidia borne singly on sharp-pointed sterigmata, fusiform, typically three-septate, simply curved or slightly sinuous; intercalary cells Prout's brown,⁴ densely granular; terminal cells lime green,⁴ less granular than intercalary cells; apical cell frequently longer and less acutely pointed than basal cell; spores occasionally constricted at middle septum, 9.9μ to 14.2μ by 29μ to 49μ , mostly 11μ to 12.5μ by 34μ to 40μ . Habitat, on leaves and culms of *Oryza sativa* (fig. 3 and fig. 1, D) and *Zizaniopsis miliacea*.

ASCIGEROUS STAGE

Perithecia dark, globose, embedded in outer tissues of sheath, 202μ to 481μ , average diameter 381μ , beak rather short (30μ to 70μ), frequently half the diameter of perithecium in width. Beak nonprotruding, tip flush with surface of outer epidermis of sheath, visible to naked eye; asci narrowly clavate, walls almost invisible and deliquescent by the time spores mature, short-stalked, 90μ to 128μ by 12μ to 14μ , mostly 103μ to 125μ by 13.5μ ; ascospores biserial, normally eight in ascus (rarely only four), three-septate when mature, usually somewhat constricted at septae, particularly at middle septum, brown, two end cells usually lighter in color and contents less granular than middle cells, fusiform, somewhat curved, 38μ to 53μ by 7μ to 8μ , mostly 44μ to 48μ by 8μ (figs. 5 and 6). Habitat, in sheaths of *Oryza sativa*.

HOSTS AND GEOGRAPHIC DISTRIBUTION

The sclerotial and conidial stages of *Leptosphaeria salvinii* occur on all commercial varieties of rice, red rice, and *Zizaniopsis miliacea* in the rice-growing areas of Arkansas, Louisiana, and Texas. The sclerotial stage occurs in most foreign countries where rice is grown. The conidial stage is known to occur only in Italy and the United States, but no doubt it is present in the other countries where the disease occurs. The ascigerous stage has been found on seven commercial varieties of rice in Arkansas, Louisiana, and Texas, on a rice selection (C. I.⁵ no. 4559) in Arkansas and Louisiana, and on commercial rice in Italy.

⁴ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D.C. 1912.

⁵ C. I. denotes accession number of Division of Cereal Crops and Diseases.

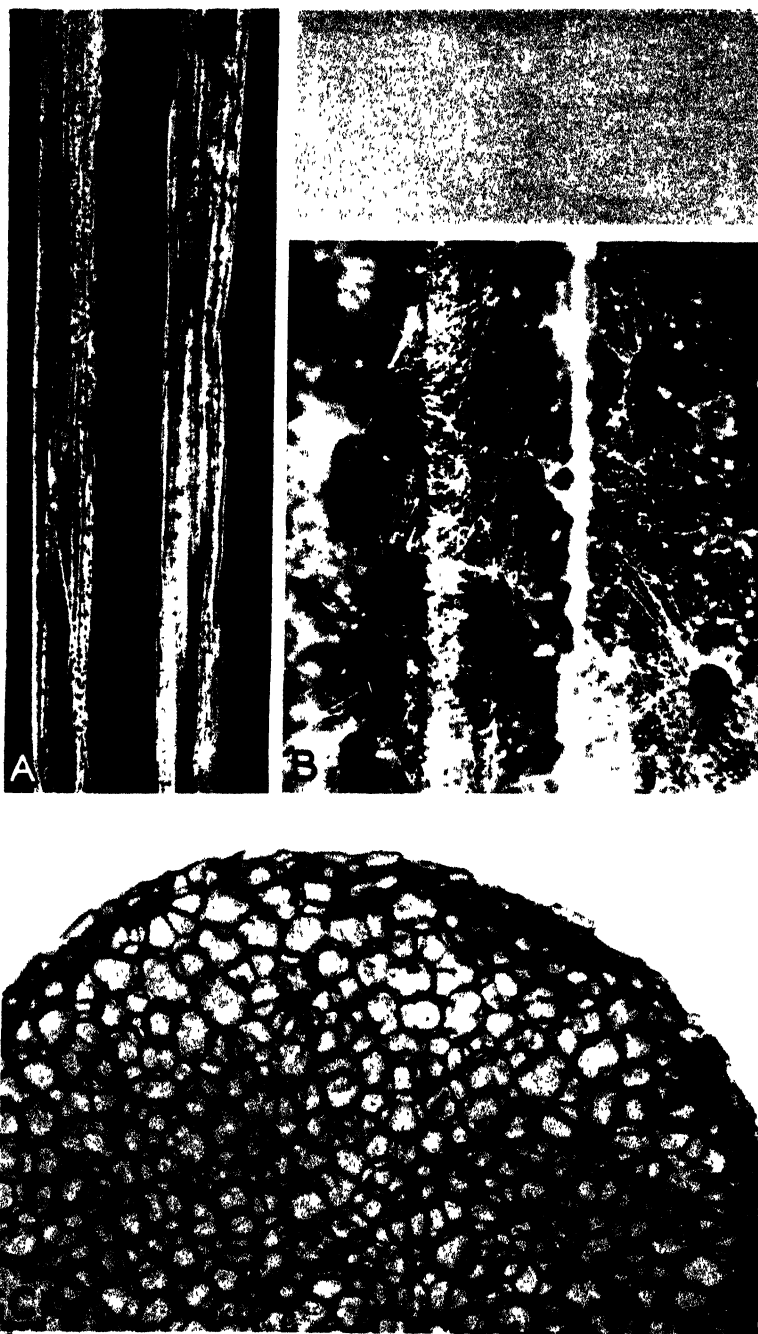


FIGURE 4.—*Leptosphaeria salicincta* sclerotia (*Sclerotium oryzae*): A, Sclerotia inside Blue Rose rice culm from near Beaumont, Tex. $\times 3$. B, Sclerotia inside Blue Rose rice culm from near Beaumont, Tex. $\times 32$. C, Portion of a cross section of a sclerotium. $\times 630$.

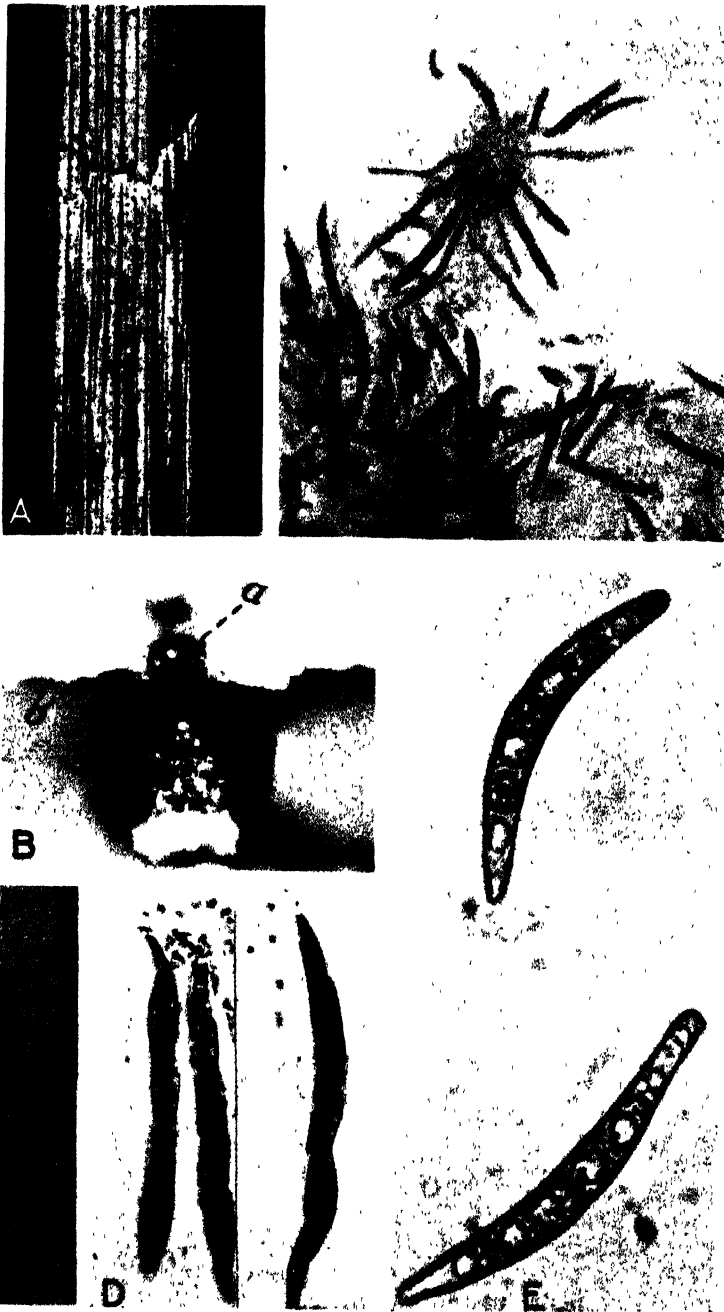


FIGURE 6.—*Leptosphaeria salvinii*, perithecial stage. A, Surface view of a portion of a sheath of Baxoro rice, showing the tips of the beaks of the perithecia appearing as small black specks. $\times 3\frac{1}{4}$. B, Perithecium showing tip of beak (a) flush with epidermis of sheath (b). $\times 50$. C, Asci in various stages of development. $\times 320$. D, Asci showing biserial arrangement of spores. $\times 132$. E, Ascospores. $\times 960$.

SEASONAL DEVELOPMENT

As Tisdale (18) has pointed out, the sclerotia are known to overwinter on old rice straw and stubble in the field and to serve as inoculum for the growing rice plants during the following summer. Indications are that these overwintered sclerotia also produce abundant conidia while floating on the surface of flood water. These conidia are readily disseminated on the surface of the water and come in contact with the sheaths and there germinate and cause infections. On the infections, whether originating from sclerotia or conidia, secondary conidia develop, which serve to spread the infection still further. Sclerotia are formed in abundance in all the infected tissues later in the season. Under certain conditions the perithecia form in the infected sheaths about the same time as the sclerotia; under other conditions they appear to form somewhat earlier.



FIGURE 5.—Reproduction of Cattaneo's illustration (4, pl. XV, figs. 1-3), the legend for which may be translated as follows: "*Leptosphaeria salvinii* n. sp., fig. 1, perithecium, fig. 2, a, ascus, b, spores, fig. 3, two germinating spores."

Viable ascospores have been found on old rice stubble as late in the winter as January. It has not been possible to determine whether they live through the winter and function as inoculum for the following crop because the sheaths carrying the perithecia disintegrate so completely during the winter that no perithecia have been found in the spring.

SUMMARY

Leptosphaeria salvinii Catt. has been found to be the ascigerous stage of *Sclerotium oryzae* Catt. and of *Helminthosporium sigmoideum* Cav., and the various stages of the fungus are described and illustrated. The hosts, geographic distribution, and seasonal development of the fungus are discussed.

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SOME FACTORS INVOLVED IN APHID TRANSMISSION OF THE CUCUMBER-MOSAIC VIRUS TO TOBACCO¹

By ISMÉ A. HOGGAN

Instructor in horticulture, University of Wisconsin, and agent, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Evidence presented in recent years from various sources has shown that insects belonging to several different families and orders may be concerned in the dissemination of plant virus diseases. Among these, species of leaf hoppers, aphids, grasshoppers, thrips, and white fly have been incriminated as vectors of specific viruses.

The nature of the relationship between a virus and its insect vector is a matter of particular interest at the present time. The subject has already received considerable attention in connection with certain viruses transmitted by leaf hoppers, such as those of curly top of sugar beet (19),² aster yellows (11), and streak disease of corn (20), and also in connection with the potato leaf-roll virus transmitted by the green peach aphid (6, 17, 18). In these cases there is evidence of an intimate association between virus and insect, which appears to indicate an increase of virus within the insect's body.

Less information is available concerning the relationship involved in the transmission of typical mosaic diseases by aphids, although many virus diseases of this type are known to be disseminated by such agents. Although a certain amount of work has been done in this connection, the results so far obtained appear to be more or less fragmentary or have not been published in full.³ A need of further information on the subject arose during recent investigations of aphid transmission of certain viruses on tobacco and related plants, some phases of which have already been reported (7, 8). As a foundation for critical experiments, it became necessary to determine various factors which might influence transmission, such as the number of aphids required for satisfactory infection, the duration of infectivity of the insect, and the time taken to acquire and to transmit the infective principle. Information of this type would be of value also as tending to indicate whether a biological relationship is involved between aphid and virus or whether transmission rests upon a purely mechanical basis. The cucumber-mosaic virus, which is readily transmissible to many solanaceous hosts by various species of aphids (7, 8), formed the subject of the present investigation, and the green peach aphid (*Myzus persicae* Sulz.) was used as the aphid vector.

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² Reference is made by number (italic) to Literature Cited, p. 703.

³ Since this manuscript was prepared, the following papers relating to this subject have been published: BENNETT, C. W. FURTHER OBSERVATIONS AND EXPERIMENTS WITH MOSAIC DISEASES OF RASPBERRIES, BLACKBERRIES, AND DEWBERRIES. Mich. Agr. Expt. Sta. Tech. Bull. 125, 32 pp., illus. 1932.

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REVIEW OF LITERATURE

That aphids may act as vectors of plant virus diseases was first recognized by Allard (1) who reported the transmission of a mosaic disease of tobacco by *Myzus persicae* and *Macrosiphum tabaci* Perg. Since this discovery a number of other virus diseases have been shown to be disseminated by one or more species of aphids. Although many reports have been limited to proof of the ability of certain species to transmit particular viruses, some have included a study of various details involved in transmission. Only the latter type of report will be considered in the present connection. The diseases concerned may perhaps be divided tentatively into two groups, namely, those of a mosaic type, such as spinach blight, cucumber mosaic, and potato mild mosaic, and those not of a true mosaic type, such as raspberry leaf curl and potato leaf roll.

McClintock and Smith (13), in an investigation of the transmission of spinach blight by *Myzus persicae* and *Macrosiphum solanifolii* Ashm., were able to obtain infection by using two or more aphids to a plant. According to the data presented, some infection resulted after the aphids had been allowed to remain on the diseased host for only 10 minutes, and transmission to healthy plants was effected after the aphids had remained on them for 5 minutes. Higher percentages of infection were secured, however, when longer feeding periods were allowed. These authors state that the lower instars were less infectious than the adults, that the aphids were able to infect several plants in succession, and that their infective capacity was not lost after molting. They also present evidence believed to indicate a direct transmission of the virus from parent aphid to offspring up to the fourth generation.

Doolittle and Walker (5), in a study of the transmission of cucumber mosaic on cucumber by *Aphis gossypii* Glov., report that the aphids were able to acquire and to transmit infection after feeding periods of 5 minutes in either case, and that they nearly always lost their infectivity after once feeding on a healthy plant. They also found that the aphids did not transmit the disease after 6 to 8 hours' confinement in a glass tube, a period said to be approximately equal to the length of time that expressed juices of diseased cucumber plants remain infectious. They state that it appears that aphid transmission of mosaic results from the virus being carried into the plant tissues on the insect's proboscis and that the minute amount of virus thus carried is exhausted during the first feeding period. So far as the writer is aware, however, these authors have not published in full the data upon which their conclusions are based.

In tests of the transmission of potato mild mosaic by *Myzus persicae*, Schultz and Folsom (14) obtained infection occasionally by using only 1 or 2 aphids per plant and more frequently by using larger numbers of insects. Storey and Bottomley (21) report a similar result in the transmission of rosette disease of peanuts by *Aphis leguminosae* Theob., single aphids only occasionally causing infection.

In a study of raspberry leaf curl transmitted by *Aphis rubiphila* Patch, F. T. Smith (16) found no evidence of transmission of the virus through the egg or to the living young. Bennett (3), in a more detailed investigation of the same disease, likewise states that the virus did not appear to be transmitted through the egg. Occasional

infection was obtained by means of single aphids, while 10 individuals were found to be as effective as 50 in causing infection. The insects became infective after 2 hours on a diseased plant and remained so up to 16 days afterward. In a later paper (4) this investigator states that all forms of the insect, including the winged form, were able to transmit the disease.

Elze (6), investigating the transmission of leaf roll of potato by *Myzus persicae*, reports an "incubation period" of the virus in the insect of 24 to 48 hours between the time of feeding on a diseased potato plant and the development of the infective capacity. The aphids retained their infectivity after remaining for 7 to 10 days on a nonsusceptible host (spinach); the virus was not lost after the insects molted and was not transmitted to the young. In the transmission of other potato virus diseases by various species of aphids neither the number of insects nor their stage of development were found to have much influence on the amount of infection secured.

K. M. Smith (17) likewise found no evidence of transmission of the potato leaf-roll virus from infective parent aphids to their progeny. The aphids retained their power of infection after feeding for 1 to 2 days on an immune host (cabbage), as well as after starvation for 4 to 5 days. Occasional infections were obtained by means of single aphids and maximum infection by means of 12 to 18 individuals per plant. This investigator also found evidence of an "incubation period" in the insect, which he states was not less than 54 hours under the conditions of the experiments (18). The aphids became infective after remaining for 6 hours on a diseased plant, though apparently not after 2 hours, and transmission of the virus was effected after 2 hours on a healthy plant. When viruliferous aphids were transferred to a fresh healthy plant every 24 to 48 hours, several, though not all, of the plants in any one series became infected.

MATERIALS AND METHODS

The cucumber-mosaic virus used in the present experiments was derived from the same source as that employed in previous investigations (7, 8), being identical with Johnson's cucumber virus 1 (10), and no doubt the same as that studied by Doolittle and Walker (5). Young tobacco plants infected with this virus by means of artificial inoculation with extract from diseased plants served as sources of infection for the aphids. All transfers of aphids were made to tobacco, except in two experiments where young cucumber plants were also used.

Virus-free colonies of the peach aphid, consisting of winged and wingless viviparous females, were maintained on healthy young cabbage plants in a large insect cage. Smaller cages, such as the one shown in figure 1, B, were generally used for confining aphids on infected plants. These were constructed of a wooden framework and had two sides of glass and two sides and the top of cheesecloth, thus allowing fairly satisfactory light and ventilation within. In order to confine aphids to single plants, glass lamp chimneys, the tops of which were covered with cheesecloth, were placed over the plants set in 5-inch pots, and the lower edge was pushed down into the soil (fig. 1, A). These proved satisfactory when placed in a good diffuse light and shaded from direct sunlight. All insect cages were kept in a greenhouse at a

temperature of about 70° F. At the end of an experiment the aphids were destroyed by fumigation with nicotine dust or in some instances were removed from the plants by means of a camel's-hair brush. The plants were then set in a warmer greenhouse (about 85°) and observed for signs of infection. The tobacco plants required for the experiments were grown in the warmer greenhouse and were kept free from aphids by means of frequent fumigation.

Two methods were employed in transferring aphids from plant to plant. Whenever feasible, leaves or portions of leaves bearing the required number of aphids were removed from the infected host and transferred to a piece of paper placed on the test plant, the aphids being allowed to migrate to the new host at will. This method, which

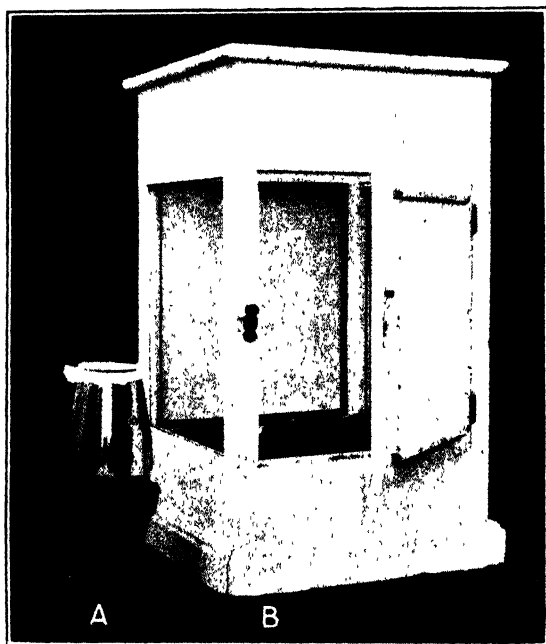


FIGURE 1.—Types of cage used for confining aphids. A, Lamp chimney, with cheesecloth top, enclosing a single plant; B, larger cage, having room for five plants in 4-inch pots.

will be referred to as the "leaf method of transfer", nearly always yielded consistent and satisfactory results. It was not always applicable, however, as, for example, in experiments where the aphids were to remain for only a given length of time on a certain plant before being moved to another. In such cases the aphids were transferred by means of a camel's-hair brush slightly moistened at the tip. Particular care was exercised in handling the aphids in order to avoid injuring them in any way. Before removal they were disturbed by a light touch with the brush, which caused them to withdraw their stylets from the plant tissues. After transference, they continued to feed and to function apparently normally and showed no signs of injury. Nevertheless, for some obscure reason the brush method of transfer proved much less reliable than the leaf method. At times satisfactory infection would be secured by this method, whereas at other times,

after an identical procedure, no infection would result, even in direct transfers of aphids from diseased to healthy plants in a manner previously found to yield infection. Consequently, in experiments necessitating the use of the brush method of transfer, individual trials in which no infection was obtained throughout were not regarded as significant if subsequent repetitions of the trial under apparently similar conditions yielded definite infection, and such individual negative trials have not been recorded in the tables. On the other hand, trials that consistently yielded negative results were of course regarded as significant. As a result, the actual numbers of infections recorded in different types of experiments are not considered to be strictly comparable; they should be taken to indicate a qualitative rather than a quantitative relationship.

In experiments involving the use of the brush method of transfer a series of plants was treated as a control by touching them with the brush as if in the process of transferring aphids to or from the plants, in order to test whether infection was carried on the brush itself. When the leaf method of transfer was used, control plants were treated by placing diseased leaves free from aphids on a piece of paper resting on the plant. Each experiment was usually repeated three times, five plants being tested in each trial for each type of aphid transfer; all conclusions were therefore based on the number of infections secured on sets of 20 plants.

EXPERIMENTAL RESULTS

The first experiments of the present investigation were more or less preliminary in nature and were designed to test the effect on the infectivity of the aphids of various factors, such as the number of insects used, their stage of development, the length of feeding time on the infected host, etc. Later experiments were directed toward determining so far as possible the nature of the association between the virus and the aphid vector. Such questions as the duration of infectivity of the aphids and the time taken to develop the infective power were specially considered. The possibility of direct transmission of the virus from infective adults to their progeny was also investigated in connection with similar tests with another species of aphid reported in another paper (9).

INFLUENCE OF NUMBER OF APHIDS ON AMOUNT OF INFECTION

Aphids were allowed to feed for 6 days or more on tobacco plants affected with mosaic and were then transferred by the leaf method to healthy young plants under lamp chimneys, in the following numbers per plant: 1, 2, 5, 10, and 20. Transfers were made to both tobacco and cucumber, wingless adults or older nymphs being used throughout. After a week or more on these plants, the aphids were destroyed by fumigation.

When only 1 or 2 aphids were transferred to each tobacco plant, no infection was obtained; some infection resulted from the transfer of 5 aphids to each plant, and increasing percentages of infection from the transfer of 10 and 20 aphids, respectively, an average of 80 percent infection being secured by the use of 20 insects per plant (table 1). Similar results were obtained on cucumber, though on this host the incidence of infection was somewhat greater throughout, 1 and 2 aphids causing infection in single cases.

TABLE 1.—*Influence of number of aphids on amount of infection secured on tobacco and cucumber*

Number of aphids per plant	Tobacco						Cucumber			
	Number of plants infected of 5 tested in experiment no. —				Total plants infected		Number of plants infected of 5 tested in experiment no.—		Total plants infected	
	1	2	3	4			1	2		
					Number	Percent			Number	Percent
1.....	0	0	0	0	0	0	1	0	1	10
2.....	0	0	0	0	0	0	0	1	1	10
5.....	1	1	2	1	5	25	2	2	4	40
10.....	2	2	3	4	11	55	4	3	7	70
20.....	4	2	5	5	16	80	5	4	9	90
0 (control)	0	0	0	0	0	0	0	0	0	0

In order to test whether single aphids were capable of infecting tobacco, a further experiment was performed in which single individuals (wingless adults or older nymphs) were transferred from a diseased host to each of a number of tobacco and cucumber plants. Of a total of 200 tobacco plants thus tested, 13 developed mosaic; that is, an average of 1 aphid in 15 or 16 caused infection. In similar trials on cucumber, the percentage of plants infected was still higher, 14 infections being secured on 100 plants, suggesting greater susceptibility of this host to aphid infection with the cucumber-mosaic virus.

These results indicate either that every aphid does not necessarily carry infection after feeding on a diseased plant, or else that every aphid does not effect successful inoculation of the plant with the virus that it carries. Possibly both conditions obtain. Since single individuals only occasionally caused infection, it became necessary to use larger numbers in order to insure more satisfactory results, and, as a rule, 20 aphids per plant were used.

EFFECT OF CERTAIN FACTORS ON INFECTIVITY OF APHIDS

STAGE OF DEVELOPMENT

In the preceding experiments only wingless adults or older nymphs were used, and it was not known whether the different forms and instars were equally effective in transmitting the virus. Comparisons were therefore made of the infective capacity of aphids in various stages of development. Since, however, it was decided to use a large number of insects per plant, it was not practicable to determine the actual instar to which each nymph belonged. The nymphs were therefore selected according to size and general appearance and classed as older, intermediate, or very young. No attempt was made to differentiate between nymphs that would develop into winged adults and those that would be wingless, although the majority were of the apterous type. Tests were made also with wingless adults. In this experiment the brush method of transfer was used, and it will be seen from table 2 that decidedly lower percentages of infection were obtained throughout, for the same number of aphids per plant, than in the preceding experiment where the leaf method was used

(table 1). Nevertheless, it is evident that all types of insect were able to transmit the disease, and no significant difference in infective capacity was observed between any of the forms tested.

TABLE 2.—*Infectivity of aphids in various stages of development*

Number of aphids per plant and developmental stage	Number of plants infected of 5 tested in experiment no.—				Total plants infected	
	1	2	3	4	Number	Percent
20 wingless adults.....	0	2	3	2	7	35
20 older nymphs.....	3	2	2	1	8	40
20 intermediate nymphs.....	1	3	2	3	9	45
20 very young nymphs.....	3	3	0	2	8	40
0 (control).....	0	0	0	0	0	0

The experiment just described showed indirectly that the aphids were able to develop the infective power after feeding on a diseased plant while they were in the nymphal stage. It did not conclusively show, however, whether they were able to acquire infectivity while in the adult stage, since the aphids tested had remained for several days on the mosaic host before transference, and the adults might have reached maturity during or after that period. In view of recent findings that the thrips vectors of "spotted wilt" of tomato and of pineapple yellowspot do not become infective if allowed to feed on diseased plants only when in the adult stage (2, 12), it seemed desirable to test whether a similar condition prevailed in the present connection. Wingless adults were therefore transferred by brush from the virus-free stock colony maintained on cabbage to tobacco plants affected with mosaic, and after 24 hours were removed to healthy tobacco plants, where they were allowed to remain for several days. A similar test was made at the same time with medium-size nymphs, probably of the second or third instar. Further trials were made with winged adults. On account of the relative scarcity of these in the stock colonies, however, the tests with this form were not always conducted at the same time, so that the percentages of infection obtained in the respective series are not strictly comparable. As a control, aphids from the virus-free stock colony were transferred directly to healthy tobacco plants. The results, which are presented in table 3, demonstrate that both winged and wingless adults were able to develop the infective capacity after feeding on a diseased host only in the adult stage.

TABLE 3.—*Ability of adult aphids to acquire infectivity*

Number of aphids per plant and type	Number of plants infected of 5 tested in experiment no.—				Total plants infected	
	1	2	3	4	Number	Percent
20 wingless adults.....	2	4	3	3	12	60
20 winged adults.....	3	4	2	0	9	45
20 nymphs.....	2	3	4	4	13	65
Many, various types from virus-free stock colony.....	0	0	0	0	0	0

FEEDING PERIODS ON DISEASED AND HEALTHY PLANTS

In the preceding experiments, before being tested for infectivity the aphids were usually kept for several days on the host serving as the source of mosaic virus, and after transference to healthy plants remained on these for several days before being destroyed by fumigation. Tests were now made to determine the time required on the mosaic host for the aphids to acquire the infective capacity, and the time required on a healthy plant for them to transmit infection. In the first case, aphids from the stock colonies were allowed to remain for different lengths of time on mosaic plants and were then transferred by brush to healthy plants, where they remained for several days. Apterous adults and older nymphs were used, 10 aphids to each plant. Tests were made of the following periods on the mosaic host: 5 minutes, 15 minutes, 1 hour, and 24 hours. A direct transfer of aphids was made from the stock colony to healthy tobacco plants, as a test for noninfectivity of insects from this source. As shown in table 4, some infection was obtained with aphids that had remained on the mosaic host for only 5 minutes, and as much infection resulted after 15 minutes on the mosaic host as after 24 hours. The lower percentage of infection secured after the 5-minute period may no doubt be attributed, in part at least, to the fact that the aphids usually took some time to settle down on the plant and probably a certain number did not feed on the host during this short stay. It is evident, nevertheless, that they were able to become infective after a feeding period as short as 5 minutes on the mosaic host.

TABLE 4.—Time required on host plants affected with mosaic for aphids to acquire infectivity

[In each test 10 aphids were placed on each plant]

Time on mosaic host	Number of plants infected of 5 tested in experiment no				Total plants infected	
	1	2	3	4	Number	Percent
5 minutes	0	2	1	1	4	20
15 minutes	4	1	2	3	10	50
1 hour	2	1	2	3	8	40
24 hours	1	4	3	1	9	45
0 (control)	0	0	0	0	0	0

In another experiment a method was adopted similar to that employed in the preceding experiment except that the aphids were allowed to remain on the mosaic host for several days and on the healthy plants for the following periods: 5 minutes, 15 minutes, 1 hour, and 24 hours. The aphids were transferred by the brush method and were removed by the same means from the test plants at the end of the experiment. Infections were obtained in all series except the controls. The average percentage of infection obtained after the 5-minute period on the healthy plants was almost as high as that obtained after the 24-hour period (table 5), indicating that the aphids were able to transmit infection to a plant almost as soon as they fed upon it.

TABLE 5.—*Time required on healthy plant for aphids to transmit infection*

Number of aphids per plant	Time on healthy plant	Number of plants infected of 5 tested in experiment no.—				Total plants infected	
		1	2	3	4		
20. ---	5 minutes ---	5	2	1	3	Number	Percent
20. ---	15 minutes ---	5	3	3	1	11	55
20. ---	1 hour. ---	3	2	3	1	12	60
20. ---	24 hours. ---	5	5	1	2	9	45
0 (control)		0	0	0	0	13	65
						0	0

TIME REQUIRED FOR DEVELOPMENT OF INFECTIVITY OF APHIDS

The preceding experiments showed that the aphids were able to acquire and to transmit infection after very short feeding periods on mosaic and healthy plants, respectively, but gave no indication as to whether the aphids were infective immediately after first feeding on a mosaic host. Since there appears to be a delay in the development of the infective power of the insect vector of certain virus diseases, commonly termed an "incubation period" of the virus within the insect, tests were made to determine whether there was a similar period of noninfectivity of the peach aphid immediately following the first feeding period on a plant affected with cucumber mosaic.

Aphids from the virus-free stock colony were transferred to mosaic plants for 15 minutes and were then moved immediately to healthy plants for a similar period, at the end of which they were removed by means of the brush and killed. Similar tests were made in which the feeding periods were increased to 1 hour on either host, and to 1 day on the mosaic host and 3 days on the healthy plants. As a control, aphids were transferred from the virus-free stock colony to healthy plants, where they remained for 4 days. The results of these tests are presented in table 6. Infection was secured in all tests in which the aphids had first fed on a mosaic host, as much infection occurring after 15-minute periods on each host as after a total period of 4 days. Hence it is clear that the aphids were able to go through the entire process of acquiring the infective principle and transmitting infection during a period of not more than 30 minutes. It is evident that no appreciable incubation period is involved.

TABLE 6.—*Infectivity of aphids immediately following a short feeding period on a host affected with mosaic*

[In each test 20 aphids were placed on each plant]

Time on mosaic host	Time on healthy host	Number of plants infected of 5 tested in experiment no. —				Total plants infected	
		1	2	3	4		
15 minutes ..	15 minutes. ---	3	1	3	3	Number	Percent
1 hour. ---	1 hour. ---	3	2	2	2	10	50
1 day. ---	3 days. ---	2	2	4	2	9	45
0 (control) ..	4 days. ---	0	0	0	0	10	50
						0	0

DURATION OF INFECTIVITY OF APHIDS

Experiments were next conducted to determine the length of time the aphids were able to retain their power of infection. Again apterous adults or older nymphs were used and were transferred by the brush method.

EFFECT OF TRANSFERRING APHIDS TO A SERIES OF HEALTHY PLANTS

After remaining on the mosaic host for about a week, 20 aphids were transferred to each of five plants, on which they were left for 2 hours. Each group of 20 aphids was then transferred to a second plant for 2 hours, and so on, up to 4 or 5 transfers. In each test some infections were obtained on the first set of plants to which aphids were transferred from the mosaic host, a total of 10 plants out of 20 becoming diseased; but in no case were any infections secured on succeeding plants. This indicated that the aphids had lost their infectivity after 2 hours on a healthy host. Control plants remained healthy when touched with the brush as if aphids were being transferred to them from the mosaic host, indicating that infection was not carried on the brush.

It seemed possible, however, that in spite of all precautions the aphids might have become injured in some way during successive transfers, and that this might account for their failure to infect any but the first plant to which they were transferred. Accordingly, a further experiment was performed in which successive transfers of aphids to healthy plants were compared with alternate transfers to healthy and to mosaic plants. In one series, aphids were transferred from a mosaic plant to healthy plants and subsequently transferred every 24 hours to fresh healthy plants; in a second series aphids were transferred every 12 hours alternately to healthy and to mosaic plants (table 7). In the successive transfers to healthy plants, as in the preceding experiment, infections were secured only on the first set of plants tested. In the alternate transfers, on the other hand, some infections were obtained in every transfer to healthy plants. The diminishing numbers of infections obtained in succeeding transfers in this second series were probably due to the gradual loss of a certain number of aphids during the experiment. When the time came to transfer the aphids to another plant, any individuals found on the lamp chimney, or elsewhere away from the plant itself, were discarded, since it was doubtful whether these had fed on the host in question. At the end of the test, therefore, the number of aphids might be considerably reduced. The experiment indicates, nevertheless, that the loss of infective power of the aphids in successive transfers to healthy plants was probably not due to any injury sustained in handling. It might be due either to a direct loss of virus resulting from the aphid's feeding on a healthy plant, or to inactivation of the virus as a result of prolonged association with the insect. Whatever the cause of loss of infectivity, the infective capacity can clearly be reacquired by a further feeding on a mosaic plant.

TABLE 7.—*Infectivity of aphids when transferred from a host affected with mosaic to a series of healthy plants, as compared with infectivity of aphids when transferred alternately to healthy and to mosaic plants*

Experiment no.	Number of aphids per plant	Number of plants infected of 5 tested										Controls
		Consecutive transfers ^a					Alternate transfers ^b					
		1 ^c	2 ^d	3 ^d	4 ^d	5 ^d	1 ^c	2 ^e	3 ^e	4 ^e	5 ^e	
1	25	3	0	0	0	0	4	1	1	0	1	0
2	30	4	0	0	0	0	3	2	1	2	2	0
3	20	4	0	0	0	0	2	2	1	1	1	0
4	20	2	0	0	0	0	2	4	1	2	2	0
Total plants infected												
Number		13	0	0	0	0	11	9	4	5	3	0
Percent		65	0	0	0	0	55	45	20	25	30	0

^a Aphids transferred every 24 hours to fresh healthy plants.^b Aphids transferred every 12 hours alternately to healthy and to mosaic plants.^c Aphids transferred from mosaic to healthy plants.^d Aphids transferred to fresh healthy plants.^e Aphids transferred to fresh healthy plants after an intervening period on the mosaic host.

EFFECT OF ISOLATING APHIDS FROM LIVING HOST

In connection with the preceding experiment, it became desirable to test the length of time the aphids would remain infective when kept away from any living host. In preliminary trials, large numbers of aphids were removed by the brush method from mosaic plants and were confined in small beakers, the mouths of which were closed by means of a piece of cheesecloth held by a rubber band. After 24 hours, the aphids were returned to healthy plants upon which they were allowed to feed. As a control, other aphids were transferred by the brush directly from mosaic to healthy plants. Although several separate tests were made, no infection was obtained in any series, whether from aphids that had been confined in beakers or from those transferred directly. The absence of infection in the direct transfers from mosaic hosts, which was contrary to expectation on the basis of previous results, was attributed to the use of the brush method of transfer, already shown to yield somewhat erratic results, since later tests involving similar transfers of aphids by the leaf method yielded fairly satisfactory infection. The experiment was therefore repeated and a somewhat different procedure followed. Leaves bearing aphids were cut from the mosaic host and enclosed in beakers until a sufficient number of insects had migrated from them. The leaves were then removed and the aphids confined in the beakers for a further period before being transferred to healthy plants. Although it was not possible to determine in this way the actual time the aphids had remained away from any source of virus, this was estimated in four separate tests to be between 18 and 27 hours. No infections resulted in any trial with aphids thus treated, although the majority survived the period of starvation and appeared unharmed when returned to healthy plants. Aphids transferred directly from the mosaic host by the leaf method, on the other hand, were highly infective. It was therefore concluded that the aphids did not retain their infective capacity indefinitely, even when prevented from feeding.

Attempts to test the infectivity of aphids after shorter intervals of starvation were not very satisfactory, because of the difficulty of obtaining sufficient migration of insects from the mosaic leaves in short periods of time. However, in two separate tests, aphids which had been kept away from the living host for a period of between 2 and 3 hours were shown to be still infective. These results suggest that the loss of infectivity of aphids following a 2-hour feeding period on a healthy plant may be due to exhaustion of the supply of virus carried by the insect rather than to any inactivation of the virus as a result of contact with the aphid, though this latter factor may perhaps account for the loss of infectivity which occurred during the longer periods of starvation.

COMPARISON OF BRUSH AND LEAF METHODS OF TRANSFERRING APHIDS

The variable results secured in experiments involving the use of the brush method of transferring aphids has been a most puzzling aspect of this investigation. It has already been pointed out that the aphids showed no signs of having been injured in any way after being transferred by this means. It has also been shown that direct transfers of aphids from mosaic to healthy plants by use of the brush method yielded at times fairly satisfactory percentages of infection, according to expectations based upon the results of previous determinations, whereas at other times the amount of infection secured fell far short of that expected. There seemed some reason to believe that transmission of the virus might be influenced to some extent by the season and the prevailing environmental conditions, though whether this was due to a direct effect upon the virus, the host plant, or the insects was entirely unknown. This subject appears to deserve further investigation. On the other hand, tests involving the use of the leaf method of transfer usually yielded consistent results. Direct comparisons have therefore been made of the two methods of transferring aphids from diseased to healthy plants, and the results of four such tests are presented in table 8. In these tests the leaf method of transfer proved much more effective than the brush method for a given number of aphids, although the reason for this difference remains obscure. Unfortunately, no other suitable method of transfer has been devised which could be substituted throughout for the brush method, so that its use has perforce been continued in experiments in which other methods were not applicable.

TABLE 8.—Comparison of the brush and leaf methods of transfer of aphids

Experiment no.	Number of aphids per plant	Number of plants infected of 5 tested		
		Brush method	Leaf method	Control (no aphids)
1.....	20	2	4	0
2.....	20	0	3	0
3.....	40	2	4	0
4.....	40	3	5	0
Total plants infected:				
Number.....		7	16	0
Percent.....		35	80	0

"INHERITANCE" OF VIRUS BY PROGENY OF INFECTIVE APHIDS

The evidence dealing with the question of "inheritance" of plant viruses by progeny of infective insects has been discussed in some detail elsewhere (9). In this connection, however, it was considered worth while to test the capacity of the peach aphid for direct transmission of the cucumber-mosaic virus from infective parent aphids to their young. Aphids were accordingly reared through several generations exclusively on mosaic-diseased plants, *Nicotiana rustica* L. being used as host instead of tobacco, since this species proved more suitable for colonizing large numbers of aphids. The young nymphs were then removed at birth by means of a fine brush and transferred to healthy tobacco plants, where they were allowed to remain for about a week. Ten nymphs were transferred thus to each of 10 plants. At the same time, apterous adults were transferred similarly to another series of plants. The adults caused infection of 5 plants out of 10 tested, while all those infested with newly-born young remained healthy. In this rather limited test there was therefore no evidence of "inheritance" of the virus by progeny of infective aphids.

With a view to making a further trial by raising colonies from infective parents on some host immune to cucumber mosaic, a search was made for an immune host that would be suitable for colonizing the insects. No such plant was found, however, and the attempt was finally abandoned. Cabbage, mustard, and turnip all proved to be susceptible to infection with the cucumber-mosaic virus by means of aphids, systemic infection apparently developing when the aphids were allowed to feed freely over the plant, and only local infection when they were restricted to single leaves. This condition is similar to that already described in connection with the infection of sugar beet with the same virus (9). In the present instance, however, no symptoms were observed on any of the plants, although the virus could be recovered from them. Nasturtium (*Tropaeolum* sp.) and pea (*Pisum sativum* L.) also appeared to develop local infection without manifesting any symptoms. Buckwheat and celery became systemically infected, showing a conspicuous mottling of the young leaves; lettuce also proved susceptible, developing a very faint, indefinite mottling. Spinach and sugar beet have been shown elsewhere to be hosts of this virus (9). Many other plants are already known to be susceptible, including many members of the Cucurbitaceae and Solanaceae, and at least one species in each of the following families: Lobeliaceae, Compositae, Martyniaceae, Asclepiadaceae, Amaranthaceae, Phytolaccaceae, and Labiatae.

DISCUSSION

The results of the present investigation on the transmission of the cucumber-mosaic virus by the green peach aphid agree in most particulars with those obtained by other investigators on the transmission by aphids of virus diseases of a mosaic type. The situation involved, however, appears to be very different from that reported in connection with certain other types of virus diseases, such as curly top of sugar beet (19), aster yellows (11), and streak disease of corn (20), which are transmitted by leaf hoppers. The absence of an "incubation period" of the cucumber-mosaic virus in the peach aphid and the failure of the insects to retain their power of infection after feeding on a healthy plant or after starvation for 18 or more hours strongly suggest that

the association between virus and insect is in this case of a mechanical rather than biological nature. The fact that the aphids are able to complete the entire process of acquiring and transmitting infection in not more than 30 minutes further suggests that the virus is probably transferred in some manner other than in the salivary glands, for it seems doubtful whether the virus would reach these organs and be ejected from them in sufficient quantity to cause infection within such a short time after being ingested. The whole situation may be explained in a manner similar to that suggested by Doolittle and Walker (5), namely, that the aphid on withdrawing its stylets from a diseased host carries with it a minute quantity of infectious plant sap, which is injected into the next plant on which the aphid feeds. In this way the aphid becomes freed from the infectious material and is rendered noninfective once more. Whether such material is carried externally or within the mouth parts, however, remains undetermined. This hypothesis is somewhat similar to that of the contamination of mouth parts recently advanced by Severin (15) to account for the short periods of transmission of the curly-top virus by the beet leaf hopper.

The process of transmission of the cucumber-mosaic virus by the peach aphid appears to differ radically from that involved in the transmission of the potato-leaf-roll virus by the same insect (6, 17, 18). In the latter disease there is evidence that the virus is carried internally by the insect and that it may multiply within the insect's body, since feeding on immune hosts does not render the insect noninfective. It is significant that these two viruses may thus be associated with a single insect apparently in such different ways. It may be noted also that there are some indications of a biological relationship between the viruses of tomato spotted wilt and pineapple yellow spot and their respective thrips vectors (2, 12).

The conclusion that the transmission of the cucumber-mosaic virus by the peach aphid is a mechanical process has a bearing on attempts to account for the selective action exhibited by this aphid in transmitting this virus but not the true tobacco-mosaic virus from tobacco plants infected with both diseases (7). If transmission is accomplished merely by a transfer of infectious material upon contaminated mouth parts, two explanations of this selective action appear possible. Either, as already suggested (8), the tobacco-mosaic virus is not extracted from those tissues of the tobacco plant on which the aphid feeds, or else some complex interaction involving virus, aphid, and plant juice occurs which rapidly renders the virus ineffective. It must be borne in mind in this connection that certain species of aphids that do not ordinarily transmit the tobacco-mosaic virus from tobacco are able to do so from tomato (8). Further information appears to be needed on the distribution of the tobacco-mosaic virus both in the various tissues of the host and within individual cells.

SUMMARY

An investigation was made of the influence of various factors on the transmission of the cucumber-mosaic virus to tobacco by the green peach aphid (*Myzus persicae* Sulz.).

Single aphids were found to cause infection only occasionally, the percentage of infection increasing with the number of aphids used.

The different forms and instars appeared to be equally effective in transmitting the virus, and adults were able to develop the power of infection after feeding on a diseased host only in the adult stage. It was shown that the aphids acquired and transmitted the infective principle after very short periods on diseased and healthy hosts respectively, and that they were able to complete the entire process in a total period of not more than 30 minutes.

Viruliferous aphids were found to have lost their infectivity after a 2-hour feeding period on a healthy plant or after starvation for 18 to 27 hours. After starvation for 2 to 3 hours, however, they appeared to be still infective.

Transferring aphids by means of a camel's-hair brush was found to be a much less satisfactory method than transferring them in situ on diseased leaves and permitting them to migrate to the new host at will.

No evidence was obtained of any direct transmission of the virus by infective parent aphids to their progeny.

The results obtained are believed to indicate that the transmission of the cucumber-mosaic virus by the peach aphid is a purely mechanical process which may be explained on the hypothesis of a transfer of infectious material upon contaminated mouth parts. The process appears to be very different from that occurring in the transmission of the potato leaf-roll virus by the same insect and from that reported in connection with the transmission of certain viruses by leaf hoppers.

It is suggested that the selective action exhibited by the peach aphid in transmitting only the cucumber-mosaic virus from tobacco plants infected with a combination of this virus and the true tobacco-mosaic virus may be due to failure of the aphids to withdraw the tobacco-mosaic virus from the diseased host.

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COMPOSITION OF EGGPLANT FRUIT AT DIFFERENT STAGES OF MATURITY IN RELATION TO ITS PREPARATION AND USE AS FOOD ¹

By C. W. CULPEPPER, *physiologist*, and H. H. MOON, *junior pomologist*, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture ²

INTRODUCTION

To make the most effective use of the eggplant (*Solanum melongena* L.) an understanding of its physical and chemical characteristics is essential. Since the fruit of the eggplant is always eaten in the immature state, it was thought best to study its chemistry at different stages of maturity in order to determine whether it would be a more desirable food at one stage than another. An effort was made to ascertain the cause of the great shrinkage that often occurs in eggplant during cooking, as well as its tendency toward excessive softening which results in an undesirable texture or consistency. A bitterness that sometimes detracts from the quality of the product and the discoloration of material that takes place during the cooking process or when exposed to air were also studied, the table quality and the food value of different varieties were compared, and the effect of differences in stage of maturity upon the palatability of the product was determined.

REVIEW OF LITERATURE

Apparently the composition of the fruit of the eggplant has not been studied extensively. The results compiled by Chatfield and Adams (4) ³ show that on an average this fruit is composed of 92.7 percent water, 1.1 percent protein, 0.2 percent fat, 0.54 percent ash, and 5.5 percent total carbohydrate, including fiber; the fuel value is given as 28.2 calories per 100 g. The percentage composition as given by Van Slyke, Taylor, and Andrews (7) agrees very closely with the values given by Chatfield and Adams. Yoshimura (8) records the presence of the nitrogenous substances adenine, trigonelline, and imidazolylethylamine in the tissues of the eggplant fruit.

Several writers (1, 3, 5) have described methods of canning eggplant. Collections of recipes for general purposes usually describe methods of preparing and cooking the material but contain little discussion of the difficulties encountered and their causes.

SOURCE OF MATERIAL

The material used in these tests was grown at the Arlington Experiment Farm, Rosslyn, Va., in 1928. The soil was a medium heavy loam of good fertility. Frequent cultivation was given to destroy weeds and

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³ Reference is made by number (italic) to Literature Cited, p. 717.

to keep the soil in good tilth. The planting included the varieties New York Improved, Long Purple, Maule Excelsior, Black Beauty, Florida Highbush, Chinese Giant, Japanese Round Purple, Ovigerum, and several foreign varieties. The fruits of these varieties differed remarkably in size, shape, and color. Ovigerum has a small white egg-shaped fruit; Long Purple has a medium-sized long purple fruit; and Black Beauty and New York Improved have very large deep-purple egg-shaped fruits.

METHODS OF SAMPLING AND ANALYSIS

To secure material of known age it was necessary to know the date of flowering of each fruit. Therefore the plots were gone over every 2 days and the flowers that had opened on the preceding day or two were tagged with the record of the date. By this method the age of the fruits could be determined within a limit of error of 1 or 2 days. Practically all the samples were collected from fruits that bloomed between August 20 and September 10. The development was therefore in the cooler part of the summer, and the rate of growth was consequently slower than if earlier flowers had been tagged. Each chemical sample was made up of sections from 5 to 10 fruits, except in the case of very young samples, when 20 or more fruits were required. The samples were taken at 5- to 10-day intervals, and sampling was continued until the fruits were 60 to 70 days old. In many cases the oldest fruits were turning yellow and the seeds were brown or black, indicating complete ripeness.

The fruits were picked between 9 and 10 a.m., brought into the laboratory, and analytical samples prepared from the unpeeled fruit by slicing radial sections from each fruit so that each sample included amounts of material from the several fruits approximately proportional to their weights. The slices were then minced finely and duplicate 100-g samples were weighed out. Enough 95 percent alcohol was added to make the concentration in the preserved material 75 to 80 percent. The samples were then heated to the boiling point of the alcohol and stored until the end of the sampling period. They were then extracted with 95 percent alcohol and the extract was made up to volume. From this fraction aliquot portions were taken for determination of soluble solids, sugars, acids, tannins, and nitrate nitrogen. After extraction the residue was dried, weighed, and recorded as the alcohol-insoluble fraction. Portions of the residue were used for the determination of the acid-hydrolyzable material.

The soluble solids were determined by drying an aliquot portion of the alcoholic extract in a vacuum oven at a temperature of 75° C. The determinations were made in triplicate, porcelain milk dishes being used for the drying of the material. The value for total solids was obtained by adding the values of the soluble and insoluble fractions.

The sugars were determined by the volumetric permanganate modification of the Munson-Walker method as given in *Methods of the Association of Official Agricultural Chemists* (2).

The acids were determined by titration with N/10 sodium hydroxide and calculated as citric acid.

The total astringency was determined by the modified Procter-Loewenthal method, and the acid-hydrolyzable polysaccharides were

determined in the residue after extraction with alcohol by boiling with hydrochloric acid. Both are described in Methods of the Association of Official Agricultural Chemists (2).

The total nitrogen was determined by the method recommended by Ranker (6) for determining total nitrogen including nitrate nitrogen. For this test a separate sample was dried in a special drying chamber with a strong current of air directed against it by an electric fan.

RESULTS OF ANALYSES

Table 1 shows the results of the analyses.

SOLIDS

It is evident from the percentage of total solids that the water content of the tissues of the eggplant is high, generally between 91 and 93 percent. The percentage of total solids in the developing

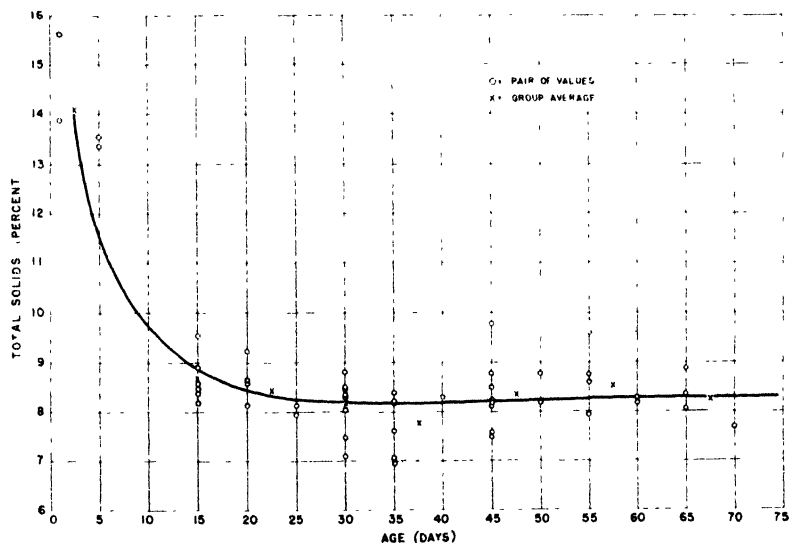


FIGURE 1 Relation of percentage of total solids to age of eggplant fruit at all stages of maturity included. Plotted from table 1; all varieties

fruit is largest at the time of flowering, when the values may be as high as 15 percent. They decrease rapidly for several days, but by the time the fruits are 15 to 20 days old a rather constant level has been reached. During the remainder of the developmental period there is no consistent tendency for the total solids either to increase or to decrease (fig. 1). From these results it is apparent that at any stage of maturity at which the fruits are likely to be used in cooking the difference in age will scarcely account for any significant difference in the solids content of the fruit.

In the 10 varieties studied there seem to be no consistent or significant varietal differences in solids content. There are striking differences in size, shape, and color of the fruit, but these do not seem to influence materially the moisture content.

TABLE 1.—Composition of fruit of different varieties of eggplant at different stages of maturity

Variety	Diameter Cm	Age Days	Soluble solids	Insoluble solids	Total solids	Reducing sugar	Sucrose	Total sugar	Titrat- able acidity	Total astrin- gency	Acid- hydrolyz- able sub- stances	Nitrate nitrogen	Total nitro- gen	Specific gravity of flesh
			Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	
Florida Highbush	0.6-0.9	1	5.27	8.62	13.89	0.51	0.72	1.23	0.207	0.288	1.256	0	0.694	
	1.0-1.2	5	5.16	8.21	13.37			1.20	0.207	0.288			0.602	
	4-5	15	4.13	4.76	8.89	1.50	1.42	2.92	0.246	0.505	1.31	0.0014	0.260	0.654
	6-8	25	4.10	3.82	7.92	2.38	3.7	2.75	0.169	0.202	1.10	0.0026	0.196	0.638
	9-11	30	4.04	3.05	7.09	2.49	4.6	2.95	0.157	0.188	0.99	0.0039	0.158	0.681
	10-12	35	4.06	3.57	7.61	2.48	1.8	2.86	0.133	0.120	1.13	0.0040	0.160	0.681
	10-12	45	4.26	3.85	8.13	2.48	6.3	3.11	0.131	0.120	1.13	0.0040	0.149	0.640
	10-12	55	4.46	3.47	7.93	2.68	.60	3.28	0.174	0.113	1.12	0.0017	0.117	0.640
	6-8	1	6.61	9.01	15.62	1.22		1.22	0.661	0.913		0	0.721	
	1-1.5	5	5.26	8.28	13.54			1.65	0.10	1.083	2.85	0	0.695	
	3-4	15	3.78	4.58	8.36	.96	.40	1.36	0.273	0.280	0.92	0.0040	0.209	0.688
New York Improved	4-6	20	3.92	4.63	8.55	1.79	.40	2.19	0.208	0.243	1.13	0.0070	0.217	0.693
	7-8	30	4.14	4.32	8.46	2.19	.29	2.48	0.182	0.243	1.29	0.0094	0.211	0.686
	8-11	35	4.10	4.05	8.15	2.50	.16	2.66	0.127	0.120	1.21	0.0091	0.191	0.614
	14-16	45	4.30	3.19	7.49	2.52	.65	3.17	0.116	0.112	0.91	0.0096	0.158	0.650
	16-18	55	4.76	3.97	8.73	2.43	1.00	3.43	0.162	0.193	1.16	0.0051	0.158	0.667
	3-4	15	3.73	4.72	8.45	.99	.45	1.44	0.260	0.432	1.04	0.0040	0.209	0.688
	4-5	20	4.36	4.86	9.22	1.56	.57	2.13	0.292	0.443	1.07	0.0056	0.215	0.696
	6-8	25	4.26	3.85	8.11	2.12	.88	2.70	0.196	0.212	1.02	0.0070	0.211	0.696
	8-10	30	4.30	3.67	7.97	2.88	.53	3.41	0.176	0.184	1.04	0.0055	0.211	0.696
	15-18	45	4.20	3.37	7.57	2.65	.44	3.09	0.130	0.105	0.90	0.0155	0.130	0.583
	18-20	60	4.18	3.99	8.17	2.12	.64	2.76	0.208	0.126	1.06	0.0097	0.126	0.580
Chinese Giant	3-4	15	4.26	3.81	8.07	2.10	.64	2.70	0.172	0.204	0.98	0.0078	0.172	0.696
	3-4	20	5.04	3.57	8.61	2.45	.64	3.09	0.240	0.240	1.13	0.0092	0.156	0.776
	8-11	45	4.82	3.12	7.94	2.92	.83	3.75	0.150	0.081	0.96	0.0144	0.112	0.700
	10-11	55	4.54	3.30	7.84	2.65	.89	3.54	0.140	0.112	1.06	0.0195	0.109	0.700
	4-5	20	4.06	3.46	7.52	2.36	.70	3.06	0.205	0.079	0.84	0.0227	0.118	0.696
	4-6	30	4.05	3.62	7.67	2.01	.69	2.70	0.215	0.118	1.16	0.0097	0.118	0.696
	4-6	35	4.14	3.93	8.07	2.06	.27	2.33	0.156	0.252	1.27	0.0012	0.130	0.696
	5-7	45	4.15	4.04	8.19	2.63	.03	2.66	0.130	0.272	1.24	0.0023	0.130	0.659
	5-7	65	4.72	3.54	8.26	2.46	.27	2.73	0.156	0.081	1.18	0.0014	0.118	0.659
	8-10	30	4.35	3.14	7.49	1.71	.71	2.48	0.175	0.091	0.91	0.0161	0.118	0.659
	10-11	35	4.48	2.75	7.23	2.81	.71	3.52	0.162	0.126	0.64	0.0027	0.105	0.563
	10-12	35	4.48	2.45	6.93	2.67	.45	3.12	0.172	0.105	0.65	0.0048	0.105	0.563
Maule Excelsior	2.5-3.5	15	5.32	4.18	9.50	3.18	.12	3.30	0.240	0.098	1.15	0.0029	0.127	0.723
	5-7	30	5.02	3.23	8.25	3.20	.86	4.06	0.169	0.081	0.99	0.0057	0.127	0.723
	6-7	40	5.34	3.31	8.65	3.31	.39	3.90	0.127	0.144	0.90	0.0195	0.127	0.723
	6-7	45	5.22	2.55	7.77	2.84	.15	3.99	0.127	0.175	1.31	0.0154	0.127	0.723
	6-7	55	5.45	2.55	8.00	2.91	.86	3.77	0.195	0.130	1.01	0.0124	0.130	0.723
	9-10	65	3.96	4.37	8.33	1.81	.66	2.47	0.223	0.128	1.02	0.0124	0.128	0.723
Japanese Round Purple	0.6-0.9	1	5.27	8.62	13.89	0.51	0.72	1.23	0.207	0.288	1.256	0	0.694	
	1.0-1.2	5	5.16	8.21	13.37			1.20	0.207	0.288			0.602	
	4-5	15	4.13	4.76	8.89	1.50	1.42	2.92	0.246	0.505	1.31	0.0014	0.260	0.654
	6-8	25	4.10	3.82	7.92	2.38	3.7	2.75	0.169	0.202	1.10	0.0026	0.196	0.638
	9-11	30	4.04	3.05	7.09	2.49	4.6	2.95	0.157	0.188	0.99	0.0039	0.158	0.681
	10-12	35	4.06	3.57	7.61	2.48	1.8	2.86	0.133	0.120	1.13	0.0040	0.160	0.681
	10-12	45	4.26	3.85	8.13	2.48	6.3	3.11	0.131	0.120	1.13	0.0040	0.149	0.640
	10-12	55	4.46	3.47	7.93	2.68	.60	3.28	0.174	0.113	1.12	0.0017	0.117	0.640
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	1-1.5	5	5.26	8.28	13.54			1.65	0.10	1.083	2.85	0	0.695	
	3-4	15	3.78	4.58	8.36	.96	.40	1.36	0.273	0.280	0.92	0.0040	0.209	0.688
	4-6	20	3.92	4.63	8.55	1.79	.40	2.19	0.208	0.243	1.13	0.0070	0.217	0.693

Ronde de Chine.....	35	4.60	3.58	5.18	2.65	.37	3.02	1.59	279	.97	.00072	670
	40	4.64	3.55	5.19	2.44	.62	4.10	1.80	144	1.04	.00037	
	70	4.70	2.99	7.69	2.86	.61	3.47	1.80	119	.78	.00121	
Noir Hative.....	35	4.44	2.61	7.05	1.60	1.15	2.75	1.82	198	.83	.00124	
	45	4.46	4.04	8.49	1.83	.76	2.39	2.41	225	1.20	.00167	
	55	4.34	4.25	8.59	1.89	.68	2.07	270	215	1.27	.00153	
	60	3.87	4.67	8.54	1.94	.43	2.37	200	160	1.55	.00087	
Ovigerum.....	30	3.86	4.92	8.80	1.98	.48	2.46	195	174	1.60	.00047	
	45	4.44	5.31	9.75	2.04	.50	2.54	225	204	1.94	.00124	
	60	3.00	5.25	8.25	1.17	.28	1.45	269	119	1.31	.00115	

On an average, approximately one half of the total solids are soluble in alcohol. The differences in the proportion of soluble to insoluble solids at different ages and in the different varieties appear to be too small to be of any practical importance in relation to the cooking quality. It seems rather remarkable that the soluble materials in this fruit should be so little influenced by the ripening processes. Many of the oldest fruits in the samples were uniformly light yellow and were therefore physiologically mature or ripe, but throughout the entire period of sampling there was very little change in soluble solids.

SUGARS

Table 1 shows that the total sugars ranged mostly between 2.0 and 3.5 percent, which, though quite low as compared with those of other fruits, nevertheless made up a considerable portion of the soluble solids. There were some differences in individual samples,

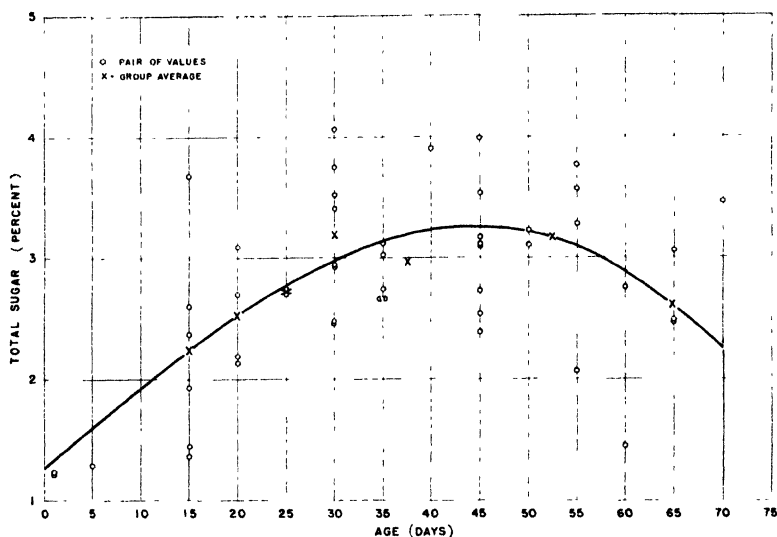


FIGURE 2 -- Relation of percentage of total sugars to age of eggplant fruit. Plotted from table 1, all varieties at all stages of maturity included

but no very marked difference in sugar content was found at any stage of maturity. There was a tendency for the sugars to increase until the fruits were 40 to 50 days old and then to decrease as they became older. This is shown in figure 2, in which the curve represents the average of all the varieties. It may be pointed out that the oldest samples of the varieties New York Improved and Florida Highbush did not decrease in sugars, but it is doubtful whether this fact is significant. The sugar content is lowest at the time of flowering, or immediately afterward, and highest when the fruits are 35 to 55 days old.

There were no very great differences in the sugar content of the different varieties; Japanese Round Purple appeared to average highest in sugar.

The principal part of the total sugars was reducing sugar, although a few samples contained as high as 1 percent of sucrose.

ACID-HYDROLYZABLE POLYSACCHARIDES

The amount of acid-hydrolyzable substances in the fruit of the eggplant was very small and there seemed to be no significant differences caused by stage of maturity or by variety.

NITROGEN

Unfortunately all the samples for the determination of total nitrogen, except those for two varieties, were lost. The total nitrogen in these two varieties indicated that the protein was very low. Total nitrogen was highest in the very young fruits, decreased rapidly for some time, then more slowly, and finally reached a constant level as the fruit approached maturity. However, the differences were too small and the number of samples too few to deter-

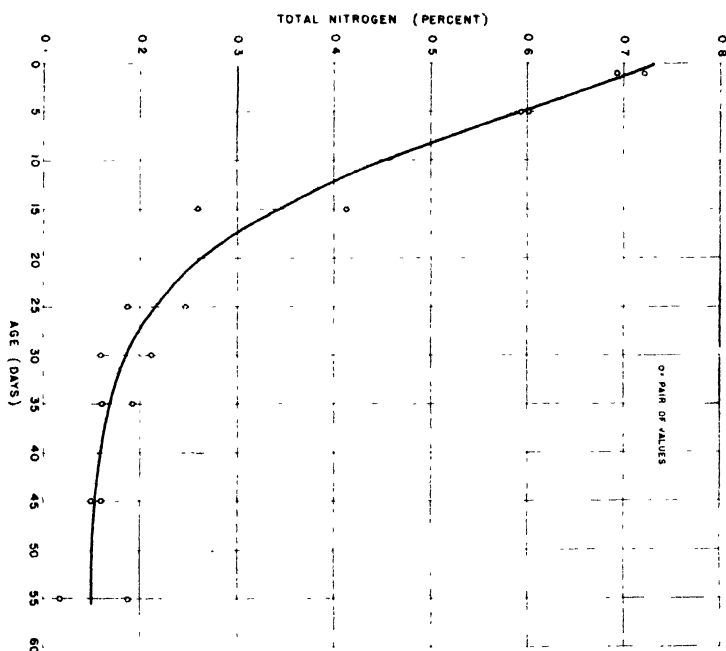


FIGURE 3 Relation of percentage of total nitrogen to age of eggplant fruit. Plotted from table 1, values of only two varieties are included.

mine with certainty the form of the curve. These data are shown in figure 3.

The percentage of nitrogen was small. If nitrates were present in the fruits at the time of flowering the quantity was too small to be measurable with the methods employed. At all ages from 15 days onward, nitrate nitrogen was invariably present and sometimes was as high as 0.0016 percent. There appeared to be no consistent differences among varieties.

SPECIFIC GRAVITY

The specific gravity was determined for a number of samples, but the results are not complete enough to indicate definitely whether there were differences due to age. It was evident, however, that

the specific gravity would be very low at any age at which the fruit would be likely to be used in cooking. The values ranged from 0.56 to 0.77, and the specific gravity of the expressed juice was 1.017, which gave a difference of 0.25 to 0.44. This indicates roughly that there was 25 to 44 percent of air by volume enclosed in the tissues. So large an amount of air in the tissues of a fruit or vegetable is rather unusual, and it might be expected that this feature would influence the behavior of the fruit in cooking.

TITRATABLE ACIDITY

The titratable acidity, calculated as citric acid, generally ranged from 0.1 to 0.2 percent in fruits 15 days old or older, but at the time of flowering a somewhat higher percentage was present. During

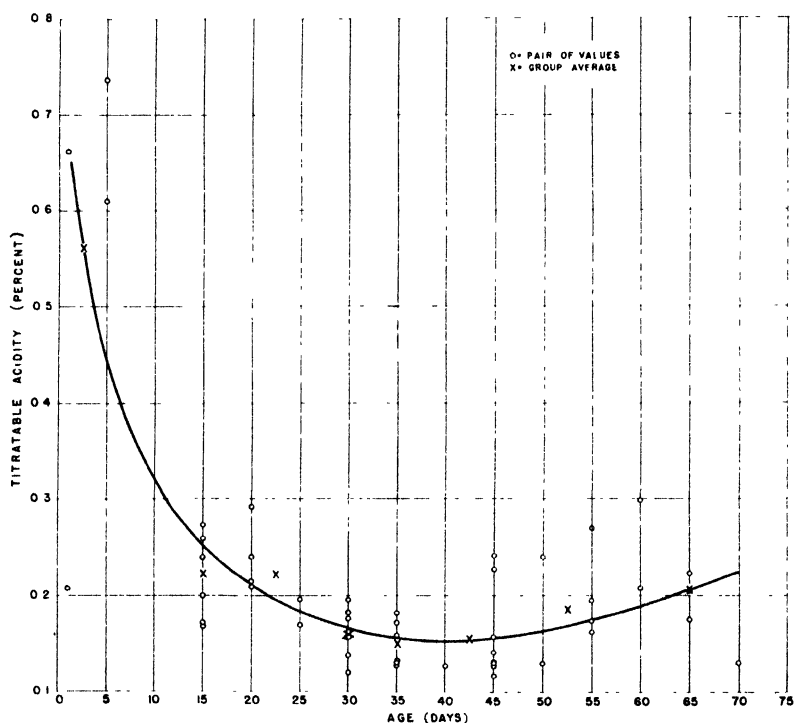


FIGURE 4 —Relation of percentage of titratable acidity to age of eggplant fruit. Plotted from table 1, all varieties included.

early development the acidity decreased slightly, reaching a low point when the fruits were 35 days of age and increasing again as they grew older. The differences, however, did not appear to be significant, at least over most of the developmental period (fig. 4).

TOTAL ASTRINGENCY

The total astringency was somewhat higher than might have been expected in view of the low total solids content. In fruits 15 days old or older total astringency in most varieties ranged from 0.15 to 0.35 percent. The total astringency was highest at the time of

flowering; for several days thereafter it decreased rapidly, then more slowly, the decrease continuing throughout the life of the fruit. There was considerable variation in individual samples, but the general trend was fairly significant. Figure 5 shows these features. The difference in total astringency among varieties seemed too small to be significant.

PECTIN CONTENT

The pectin content of the fruit was not determined quantitatively, but a number of qualitative tests were made which apparently were important. A quantity of fresh material was ground in a food chopper; the juice was pressed out immediately and strained through cheesecloth, and a portion was filtered through filter paper. The freshly expressed juice turned brown on standing in the air; it was

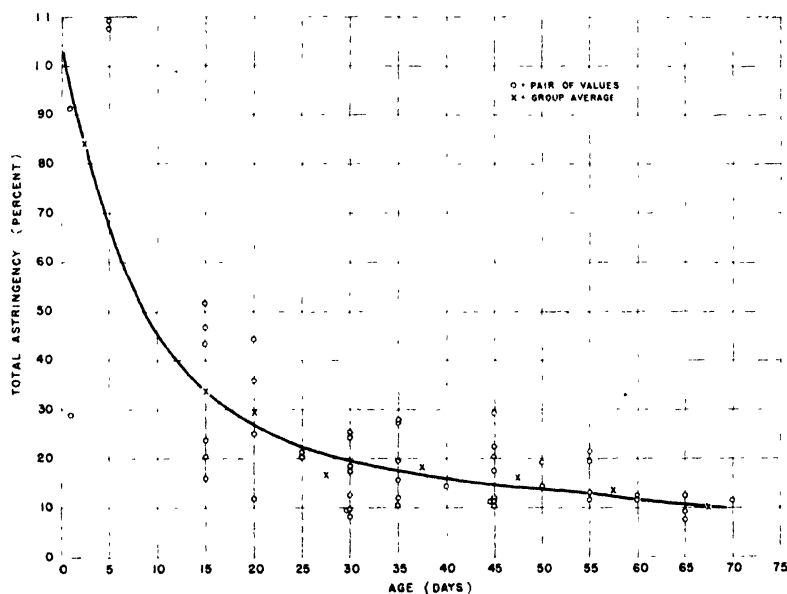


FIGURE 5 -Relation of percentage of total astringency to age of eggplant fruit. Plotted from table 1, all varieties at all stages of maturity included.

slightly viscous and gave a very slight precipitate with alcohol. A portion of the filtered juice was made alkaline with sodium hydroxide, allowed to stand overnight, then acidified with acetic acid and treated with a solution of calcium chloride. Very little precipitate was obtained. Another quantity of material was minced finely and boiled with water, forming a viscous extract which yielded a precipitate with alcohol. After this extract had been made alkaline with sodium hydroxide, allowed to stand overnight, and then acidified with acetic acid and treated with calcium chloride, the solution gave a rather heavy precipitate. Still another portion was extracted in the same way but with dilute citric acid. The extract was very viscous and could not be readily filtered through filter paper unless it was greatly diluted. The quantity of material extracted was much greater than when water was used. The extract gave a voluminous precipi-

tate with alcohol, and when treated with calcium chloride in the manner just described it gave a large amount of precipitate.

A quantity of material was boiled with dilute ammonia; this resulted in almost complete disintegration of the tissues and gave a viscous solution. Some precipitate was obtained when the solution was neutralized, and an insoluble compound was formed on treatment with calcium chloride.

These tests strongly indicate that there is a considerable amount of pectinlike material in the tissues of the fruit of the eggplant. It appears to be mostly in the form of protopectin, very little true water-soluble pectin being present.

In the course of the investigation fruits from several varieties of eggplant at various stages of maturity were steamed, boiled, or fried, in order to determine what changes take place in the cooking process. The fruits were washed, peeled, and sliced. Certain lots were salted and pressed; others were cooked without this treatment. In some recipes it is stated that salting and pressing the slices removes bitter substances. In no variety and at no stage of maturity was an objectionable bitterness found in the material used in these tests; therefore it did not seem necessary to extract the juices with salt or to soak the pieces in salt water before frying. However, the practice may have some merit, since the moisture content is very high and some of the water of the fruit may be rapidly extracted by salt or by soaking in salt water. There may be some difficulty in frying the pieces of the untreated material so that they will remain intact until served, whereas the pieces from which a part of the water has been removed will have a heavier consistency and hence a greater tendency to remain intact. The protopectin, which is largely responsible for the consistency, is not removed by the salting and pressing treatment. Of course some sugars and salts are lost, but the principal substance removed is water. If bitter substances are present they also may be partially removed. This treatment also expels a considerable portion of the air that is enclosed in the tissues.

When steamed or boiled in water the slices of the eggplant fruit became very soft and partly or wholly lost their original shape, showing considerable shrinkage. These changes were apparently due to the peculiar chemical and physical characteristics of the fruit. The softening was the result largely of the changes that occurred in the structural constituents of the tissues or to the change of protopectin into pectin as a result of heating. The high moisture content and the low starch content apparently resulted in a watery consistency which caused the material to readily lose its original shape. The shrinkage was probably due to the driving out of a large amount of the air enclosed in the tissues, which, as already stated, makes up from 25 to 44 percent of the volume of the fruit. It is evident that if water is extracted or escapes from the tissues during preparation for cooking, shrinkage results from this cause also.

The stage of maturity of the fruit seems to be of no particular importance from the standpoint of its cooking qualities. In varieties like Black Beauty and New York Improved the seeds become noticeable at the age of 35 to 40 days from the date of blooming, but even at 50 days they were not particularly objectionable. In early varieties like Ovigerum and Noir Hâtive the seeds mature considerably earlier

than in such varieties as Black Beauty and New York Improved. In these tests, fruits about two thirds grown appeared slightly superior when all points were considered. The age at which this size is reached varies with the earliness of the variety, but it was generally between 25 and 40 days under the climatic conditions under which this material was grown.

The cooking quality of the different varieties did not differ markedly and no variety seemed outstanding as compared with the others. Long Purple, Black Beauty, and New York Improved, because of the agreeable flavor and the tender quality of their flesh, were considered slightly superior to Florida Highbush and Chinese Giant. It was expected that some of the varieties would exhibit the bitterness that is sometimes complained of, but such was not the case. Since the varieties studied include those most generally grown, it seems that this trouble must be due not to the variety but to seasonal conditions or to the method of cooking. However, it must be remembered that individuals differ in their sensitiveness to unpleasant flavors. The persons judging this material were necessarily restricted to members of the writers' laboratory force. Extended tasting tests might have revealed some individuals to whom the bitterness even in this material was noticeable.

If the fruits were allowed to stand in the air after they were peeled and sliced they became somewhat brown, but the addition of salt prevented this to some extent. In practically all the cooking tests the material showed a tendency to darken, which increased somewhat when it was exposed to the air after cooking. The darkening was not sufficient to be objectionable, however, unless the material had come in contact with iron. Fruits cooked in an iron kettle became quite badly discolored. In a few tests a quantity of material that was boiled with a small amount of iron filings became greatly discolored. The discoloration was apparently due to a reaction between the iron and the tannin or other hydroxybenzene derivatives. The compounds formed promptly turn dark because of the oxidizing action of the air. The chemical analysis shows a significant amount of tannin compounds in all varieties. The low acidity also favors these reactions. The pH values of the juice ranged from 5.0 to 5.79 for the various varieties; the titratable acidity generally ranged from 0.1 to 0.2 percent. It is obvious that in order to prevent this discoloration the material must be kept from contact with iron or iron salts. The behavior of similar oxidation systems in other materials suggests that the addition of a little vinegar or lemon juice might aid in preventing any objectionable discoloration.

CANNING TESTS

No extensive canning tests were undertaken, but several lots of fruits of the varieties Black Beauty, New York Improved, Florida Highbush, and Long Purple were packed in various ways. The fruits were peeled and cut into circular disks one fourth of an inch thick and of the same diameter as the can. One lot was blanched in steam for 1 minute, packed in cans without preheating, exhausted 3 minutes, and then sealed. Another lot was prepared by thoroughly cooking the material, then pulping it and packing it while hot into the cans. In one lot the circular disks were sprinkled with salt and allowed to stand until about one third of the weight of the juice had been re-

moved; they were then packed in no. 2 cans, exhausted 3 minutes, and sealed. The juice likewise was poured into cans, exhausted 3 minutes, and sealed. All cans, regardless of their previous treatment, were processed for 1 hour at 240° F. and cooled in the air.

In several instances the product was fairly satisfactory, but on the whole the possibility of obtaining a highly attractive pack was not promising. The most satisfactory product was obtained when the material was blanched in steam and then exhausted before processing. All lots showed a tendency to darken, which seemed to be due, as already pointed out, to the presence of tanninlike compounds which reacted with the metal of the can. The reaction was favored by a very low acidity. The shrinkage was excessive unless the air was removed by pressing or exhausting. The material generally became too soft to hold its form properly. Further work would be required to develop the best method of handling the product.

There was considerable corrosion of the can similar to that caused by pumpkin. The tin was removed or dissolved, exposing the iron, which turned dark. The air or oxygen in the tissues may have been responsible for the corrosion, but the presence of nitrates may also have been an important factor. The fact that the juice alone caused practically as much corrosion as the fruit itself indicates that oxygen was probably not the only factor involved.

SUMMARY

A study was made of the composition of the fruit of the eggplant (*Solanum melongena* L.) at different stages of maturity, in relation to its behavior in cooking. The results obtained are as follows:

The total solids were low, or the moisture content high, at all stages of maturity at which the fruit would be likely to be used in cooking. The total solids generally ranged from 7 to 9 percent of the fresh green weight. They were highest in the material at the time of flowering but decreased rapidly for a few days thereafter. This decrease ceased when the fruits were 15 to 20 days old, and for the remainder of the developmental period there was usually very little change in solids.

The total sugars, which in general ranged from 2.0 to 3.5 percent of the fresh green weight, made up a substantial portion of the soluble solids. The percentage of sugar was not greatly influenced by the stage of maturity, although there was a tendency for sugar to be highest in fruit about 40 days old, or about the time when it is generally picked for table use.

The starch or other material converted into sugar by acid hydrolysis was very small in amount and differed little with stage of maturity or variety.

The percentage of total nitrogen in the material was low. It was highest in the very young fruits, decreased rather rapidly for several days, then more slowly, and appeared to reach a constant level about the time the fruits would be picked for table use. A small but significant amount of nitrate nitrogen was present at all stages of development except the very earliest.

The specific gravity of the fruits was very low; the difference in the specific gravity of the intact tissues and that of the expressed juice indicated the presence of 25 to 44 percent of air (by volume) in the tissues.

The titratable acidity was quite low at all stages of maturity. Except in the earliest stages, there was little correlation between differences in acidity and differences in age. There was a tendency for the acidity to be lowest in fruit 35 to 45 days old, or about the time when it would be used for cooking.

There was a rather significant quantity of astringent materials present in the tissues of the fruit. These were highest at the time of flowering and decreased rapidly for a few days, then more slowly until the fruit was completely mature. During the period at which the fruit would be likely to be used on the table, its age is correlated with only very small differences in the percentage of astringent materials.

The differences in the composition of the varieties studied seemed too small to be of any practical significance.

Chemical and cooking tests indicated that the failure of the material to hold its form was due to the high moisture content of the material, associated with the change of a considerable part of the protopectin into pectin during cooking. This resulted in a product that was very soft in texture; the heating had a tendency to drive out the air, causing the material to shrink.

The tendency for the material to turn dark in cooking seemed to be due to the presence of tanninlike substances associated with low acidity. Iron and salts also reacted with the tannins to form compounds that darkened in the air, hence it is obvious that care should be exercised not to bring the material into contact with iron.

In the canning process there was a tendency for the product to turn dark, become soft, and lose its form. The tin can was rather severely corroded by the material. The presence of oxygen and nitrates suggests that these may have been responsible for the action on the can.

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LOGARITHMIC EXPRESSION OF TIMBER-TREE VOLUME¹

By FRANCIS X. SCHUMACHER, *silviculturist, Forest Service, United States Department of Agriculture*, and FRANCISCO DOS SANTOS HALL, *assistant professor of forestry, Instituto Superior de Agronomia, Portugal*²

INTRODUCTION

Volume tables, i.e., tabulated average volumes of trees of given diameters and heights, are basic to the management of any forest property for which the primary purpose of management is production of wood or lumber volume.

Volume tables are made for individual timber species, and often are made separately for virgin and second growth and for other intra-species classes such as those defined by site quality or by geographical location. They show cubic volume either of the entire main stem or of that part of it between the stump and a merchantable top diameter, or board-foot volume between the stump and a merchantable top diameter, this diameter being in some cases a constant and in others a variable depending upon the size of the tree.

The general method of constructing these tables is to correlate tree volume with diameter and height. Several modifications of the general method are in use. All the methods in common use imply ignorance as to the mathematical relationship of volume to dimension; for they rely upon the free-hand curve process, either entirely³ or partially.⁴ For this reason it is practically out of the question to determine the significance of differences between volume tables for two or more species in one locality or for the same species in two or more localities; the effort of fitting free-hand curves to each of the sets of data cannot be so objective but that differences result from the personal factor involved in this operation, and these differences cannot be segregated from those due to differences in species or locality.

It is the purpose of this paper (1) to present a theory bearing on the mathematical expression of tree volumes, of the power or logarithmic type, in terms of diameter breast high (d.b.h.) and height of tree; (2) to test the theory with available data; (3) to show that volume comparisons between species and localities can be placed upon a strictly objective basis; and (4) to apply the logarithmic equations to volume-table construction.

THE THEORY AND ITS TEST

(A) CUBIC VOLUME OF THE ENTIRE STEM IN TERMS OF D.B.H. AND TOTAL HEIGHT

The fundamental relationship is that of volume of the entire stem, with or without bark, to diameter breast high (d.b.h.) and total height

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² Particular acknowledgment is due Roy Chapman, junior forester, Southern Forest Experiment Station, for valuable assistance and criticism throughout the investigation, and to Bert R. Lexon, assistant silviculturist, Southwestern Forest and Range Experiment Station, who carried on part of the early work in the investigation. The tree-volume data used were supplied by A. C. McIntyre, of the Pennsylvania State College, by the Maryland Department of Forestry, and by several of the forest experiment stations of the U. S. Department of Agriculture.

³ CHAPMAN, H. H., and DEMERITT, D. B. ELEMENTS OF FOREST MENSURATION. p. 202-212. Albany 1932.

⁴ ——— See footnote 3, p. 216-205.
REINEKE, L. H., and BRUCE, D. AN ALIGNMENT-CHART METHOD FOR PREPARING FOREST-TREE VOLUME TABLES. U. S. Dept. Agr. Tech. Bul. 304, 28 p., illus. 1932.

of tree. If the form of the stem is independent of its size, the volume equation is

$$V = D^2 H C \quad (1)$$

in which V is cubic volume of the entire stem, D is d.b.h., H is total height, and C is a constant. This equation may be analyzed by the following reasoning: Since volume is to be in cubic feet, d.b.h. in inches, and total height in feet,

$$V = \frac{\pi}{4 (144)} D^2 H F \quad (2)$$

in which F is the cylindrical form factor used in forest mensuration. Equation (2) reduces to

$$V = D^2 H (0.005454 F) \quad (3)$$

so that C of equation (1) becomes $0.005454 F$. Now if $F = 1$, equation (3) is simply the volume of a cylinder in cubic feet, when its diameter is measured in inches and its height in feet. But

$$F = \frac{V_T}{V_C}$$

in which V_T is the cubic volume of a tree and V_C is the cubic volume of a cylinder of the same height and diameter; so that if we assume the correlation of form factor with d.b.h. and height of a sample of trees to be zero, we are assuming from equation (3) that the cubic volume of the entire stem increases as the square of d.b.h. and directly as height. This, in fact, seldom holds. If, however, volume increases as certain other powers of d.b.h. and height, the volume equation becomes

$$V = D^{b_1} H^{b_2} C \quad (4).$$

Taking logarithms, we have the linear form

$$\log V = b_1 \log D + b_2 \log H + \log C.$$

If we now denote the logarithms of volume, d.b.h., height, and the constant term by X_1 , X_2 , X_3 , and K , respectively, we have the familiar form of the multiple regression equation

$$X_1 = b_{12.3} X_2 + b_{13.2} X_3 + K \quad (5)$$

in which the partial regression coefficients $b_{12.3}$ and $b_{13.2}$ are the exponents of d.b.h. and height, respectively, in equation (4).

The theory presented, then, is that equation (4) is a rational expression of the relation of the cubic volume of the entire stem to d.b.h. and height; in other words, that the relation of the logarithm of volume to the logarithms of d.b.h. and height is linear. If so, the exponents and constant term may be empirically determined for a given sample by the method of least squares, and tests of the significance of the differences between two or more samples may be applied statistically.

An equation of this type was first applied to data for 264 yellow poplar trees. The calculated statistics are given in table 1. The logarithmic regression equation from these statistics—formulated according to the method given by Yule⁵—becomes

$$X_1 = 1.7924X_2 + 1.0565X_3 - 2.5220$$

and the multiple correlation coefficient $R_{1.23} = 0.9981$. The test of the linearity of the relationship of the logarithm of volume to the logarithms of diameter and height is made by putting the equation into alignment-chart form and comparing the actual X_1 value of each tree with the estimated X_1 read from the chart. In figure 1 the result is presented graphically. The excellence of the fit establishes the linearity of the logarithmic relationship for these data.

TABLE 1.—Means and standard deviations of the logarithms of d.b.h. inside bark, total height, and volume of wood of the entire stem, and gross correlation between the logarithms of the variables, for 264 yellow poplar trees

Variable X	Mean M	Standard deviation σ	Correlation coefficient r
X_1 —logarithm of volume	Logarithm $M_1 = 0.434$	Logarithm $\sigma_1 = 0.573$	$r_{12} = +0.990$
X_2 —logarithm of d.b.h. inside bark	$M_2 = .885$	$\sigma_2 = .236$	$r_{13} = +.943$
X_3 —logarithm of total height	$M_3 = 1.770$	$\sigma_3 = 1.54$	$r_{23} = +.893$

As this logarithmic equation is linear, we may immediately write the equation of the cylindrical form factor of these trees. If F is made equal to 1, equation (3) gives the volume of a cylinder of the same diameter and height as the tree; its logarithmic form is

$$\log V_C = 2 \log D + \log H - 2.2672.$$

Since

$$\log F = \log V_T - \log V_C$$

subtraction of the logarithmic cylinder-volume equation from the logarithmic tree-volume equation gives the logarithmic form of the form-factor equation. For the sample of 264 yellow poplar trees this is

$$\log F = -0.2076 \log D + 0.0565 \log H - 0.2548.$$

It is evident, therefore, that the divergence of the d.b.h. and height exponents from 2 and from 1, respectively, measures the change of form factor per unit change of dimension.

The same type of equation was fitted to data for eight other timber species, hardwood and conifer, representative of four forest regions. In every case the comparison of the data with the equation for their species served to confirm the hypothesis that the equation type is a general one. The logarithmic regression coefficient, the constant

⁵ YULE, G. U. AN INTRODUCTION TO THE THEORY OF STATISTICS. Ed. 3, rev., p. 229-252. London, 1927.

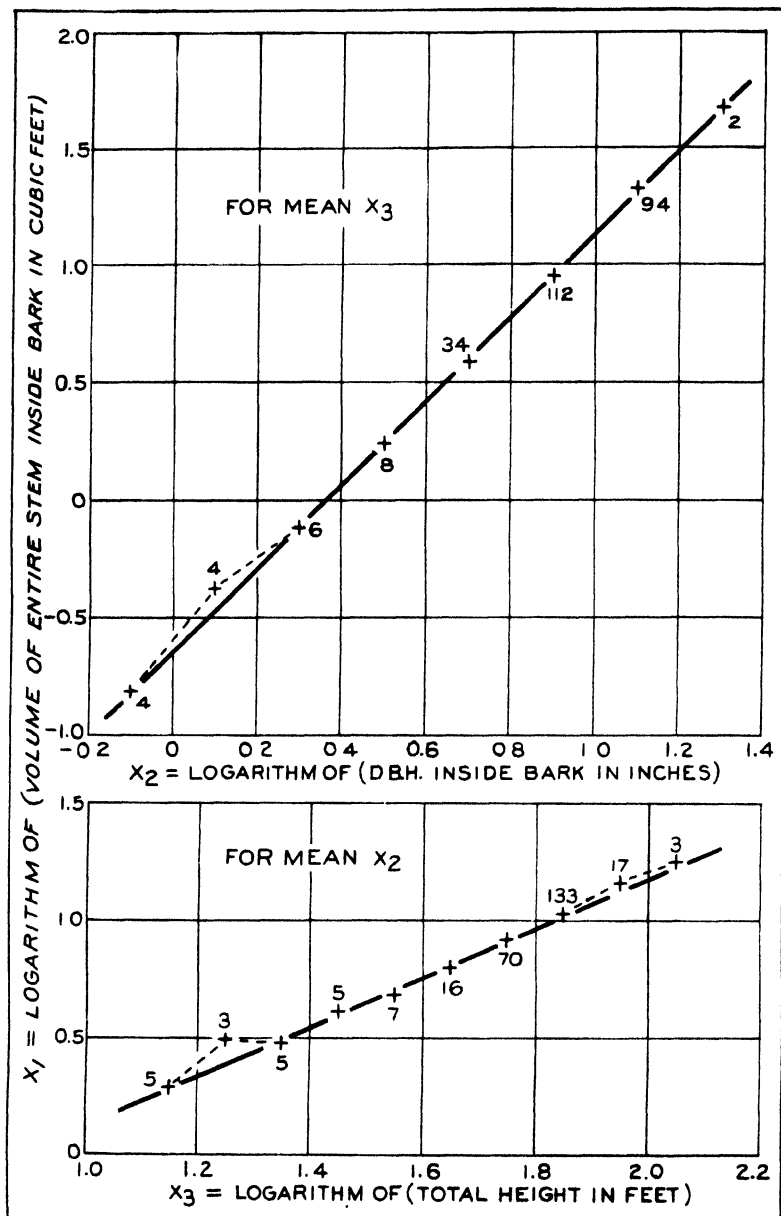


FIGURE 1 - Graphic test of the linearity of a logarithmic volume equation based on measurements of 264 yellow poplar trees. The dependent variable is the logarithm of the cubic-foot volume of the entire stem inside bark.

term, and certain statistics for the data for each of these species are given in table 2.⁶

TABLE 2.—Logarithmic regression coefficients, constant term, and certain pertinent statistics for nine timber species for which logarithmic equations of the cubic-foot volume of the entire stem inside bark were calculated

Species	Partial regression coefficients		Constant, C	Means		
	b_{12}	b_{22}		M_1	M_2	M_3
				Logarithm	Logarithm	Logarithm
Virginia pine, <i>Pinus virginiana</i>	1 7023	1 2639	-2 6030	0 7708	0 5678	1 5439
Shortleaf pine, <i>Pinus echinata</i>	1 8081	1 2345	-2 7582	1 0207	.8908	1 7622
Loblolly pine, <i>Pinus taeda</i>	1 9136	.9532	-2 3723	1 2249	.9638	1 7787
Red maple, <i>Acer rubrum</i>	1 8361	1 0191	-2 4821	4762	.6851	1 6683
Chestnut, <i>Castanea dentata</i>	1 7779	1 1302	-2 6260	8324	8423	1 7255
Douglas fir, <i>Pseudotsuga taxifolia</i>	1 6653	1 1800	-2 6681	2 9011	1 7048	2 3152
Black walnut, <i>Juglans nigra</i>	1 7173	1 1974	-2 6842	.7515	.8185	1 6954
Red gum, <i>Liquidambar styraciflua</i>	1 7470	1 1644	-2 6819	.9766	.9039	1 7858
Yellow poplar, <i>Liriodendron tulipifera</i>	1 7924	1 0565	-2 5220	9340	8852	1 7699

Species	Standard deviations			Trees, N	Standard error of estimate, σ_1	Gross correlation coefficient, r_2
	σ_1	σ_2	σ			
	Logarithm	Logarithm	Logarithm	Number	Logarithm	Logarithm
Virginia pine, <i>Pinus virginiana</i>	0 4117	0 1677	0 1173	846	0 0925	0 7383
Shortleaf pine, <i>Pinus echinata</i>	.5164	.1900	.1362	285	.0854	.8608
Loblolly pine, <i>Pinus taeda</i>	.5231	.2088	.1430	370	.0271	.8355
Red maple, <i>Acer rubrum</i>	.5791	.2485	.1206	337	.1570	.9072
Chestnut, <i>Castanea dentata</i>	.6729	.2761	.1651	704	.0851	.9268
Douglas fir, <i>Pseudotsuga taxifolia</i>	.0083	.1820	.0652	217	.0923	.7080
Black walnut, <i>Juglans nigra</i>	.6883	.2625	.1716	400	.0793	.9179
Red gum, <i>Liquidambar styraciflua</i>	.8693	.3334	.2490	388	.1154	.9627
Yellow poplar, <i>Liriodendron tulipifera</i>	.5731	.2362	.1539	264	.0367	.8931

In table 2 the logarithm of d.b.h. inside bark was taken as the diameter variable for every species but one. For the Douglas fir data this measurement was not available, so the logarithm of d.b.h. outside bark was used instead. The test for Douglas fir is shown in figure 2.

(B) MERCHANTABLE CUBIC VOLUME TO A FIXED TOP DIAMETER IN TERMS OF D.B.H. AND TOTAL HEIGHT

Not uncommonly an estimate of cubic volume between stump and a fixed top diameter of the stem is needed. Such a conception of cubic volume requires a modification of equation (4), which, however, does not introduce any new difficulty.

⁶ It is perhaps well to call attention to methods that facilitate the use of logarithms of numbers less than 1. To evade the difficulty of using negative characteristics with positive mantissas in combination with characteristics and mantissas both of which are positive, in frequency distributions and cross-products of logarithms, two methods are available: (1) To all characteristics of that variable in which a negative characteristic appears in one or more of the variates, add a constant integer sufficient to make all characteristics positive. For instance, if the smallest volume is 0.123 cubic feet, its logarithm is 1.0899, in this case add 1 to all volume characteristics. The logarithmic value of this volume is then considered to be 0.0899. Adding the constant to the characteristic does not affect the standard deviation or the product-moment, but it does affect the arithmetic mean of the logarithms, the integer must be subtracted from the mean to arrive at the true mean. (2) Transcribe those logarithms that have negative characteristics to the negative form, thus:

$$\begin{aligned} \text{Cubic volume} &= 0.123 & \log 1.0899 \\ \text{add zero (1 e, +1} & \dots -1) & +1. \\ & & \hline & 0.0899 - 1 \end{aligned}$$

and this is equal to -0.9101.

This alternative is usually the simpler procedure unless the data contain a large number of values less than 1, for no statistical constant is affected.

Let us suppose that we want an equation of cubic volume of the main stem, including bark, between a 1-foot stump and a top diameter of 4 inches. Obviously a tree must be 4 inches in d.b.h. before it contains any volume whatever by this definition; but as soon as its

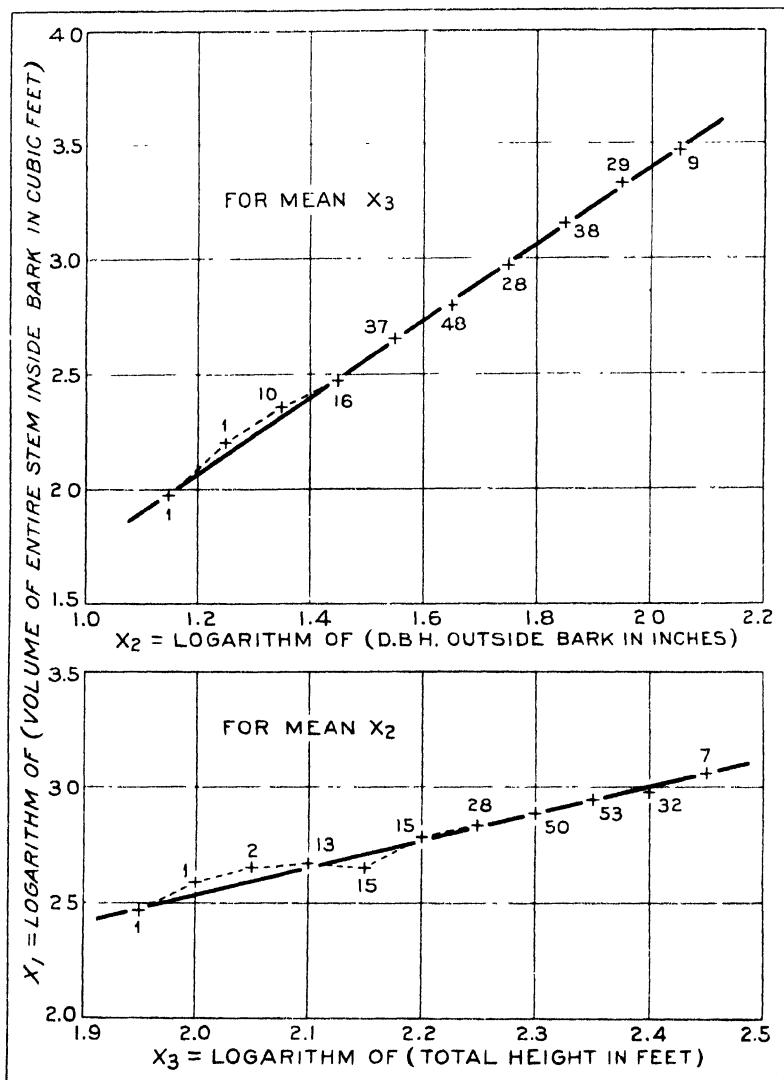


FIGURE 2.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 217 Douglas fir trees. The dependent variable is the logarithm of the cubic-foot volume of the entire stem inside bark.

d.b.h. is just 4 inches, its volume is that of a bolt 4 inches in diameter and $3\frac{1}{2}$ feet long (the length from breast height to stump). The origin of coordinates of the volume equation is therefore transferred from zero volume to the volume of this bolt (0.30 cubic foot), from zero d.b.h. to 4 inches d.b.h., and from zero height to 4.5 feet height.

Hence the equation for merchantable cubic volume corresponding to equation (4) is

$$V - v = (D - d)^{b_1} (H - 4.5)^{b_2} C \quad (6)$$

in which V is cubic volume of the stem between stump and the fixed top diameter d , v is the cubic volume of a bolt of diameter d and length equal to the distance from stump to breast height, and D , H , and C have the same meanings as before. Denoting the logarithms of $(V - v)$, $(D - d)$, and $(H - 4.5)$ by X_1 , X_2 , and X_3 , respectively, we have the multiple regression form of equation (5). Figure 3 shows the graphic test of the linearity of the relationship of the logarithms of the volume of the black walnut trees with the logarithms of their diameter and height, the logarithms of the volume being expressed by the equation

$$\begin{aligned} \log (V - 0.30) = & 0.8274 \log (D - 4) \\ & + 1.7262 \log (H - 4.5) - 2.4965. \end{aligned}$$

The merchantable volume of these trees is the cubic-foot volume in wood and bark of the stem between stump height of 1 foot and top diameter, outside bark, of 4 inches. Diameter at breast height is outside bark. The fit of the linear equation in logarithms is excellent.

(C) BOARD-FOOT VOLUME TO A FIXED TOP DIAMETER INSIDE BARK IN TERMS OF D.B.H. INSIDE BARK AND TOTAL HEIGHT

Most of the board-foot volume tables for second-growth timber list average board-foot contents between stump and a fixed top diameter inside bark, scaled according to saw-log lengths of 16 feet with the fractional log length always uppermost. Except for the change in volume unit from cubic foot to board foot, this expression of volume is the same as that of merchantable cubic volume. Equation (6) should therefore express the relationship of board-foot volume to d.b.h. and total height.

The definition of board-foot volume usually specifies, however, that a tree is considered to have board-foot volume only if its length between the stump and the fixed diameter limit amounts to at least 16 feet, that is, only if it contains at least one full saw log. Suppose we need the board-foot volume by the International log rule between a 1-foot stump and a 5-inch top diameter inside bark. Under this specification, the tree must be 5 inches in diameter inside bark at 17 feet from the ground before it is considered to have any board-foot contents. As soon as it reaches these dimensions, the origin of coordinates is transferred from zero to 5 inches d.b.h. inside bark, from zero to 17 feet height, and from zero volume to the volume of a 16-foot log 5 inches in diameter (13 board feet). Using the form of equation (6), we have

$$V - 13 = (D - 5)^{b_1} (H - 17)^{b_2} C$$

in which V is volume in board feet as defined above, D is d.b.h. inside bark, and H is total height.

This equation as it stands is misleading, in a way that is illustrated by figure 4. The two trees pictured therein are identical in d.b.h. inside bark and in total height. Tree A, containing just one full 16-foot saw log, is considered to have a volume of 13 board feet; but

tree B, containing a 13-foot saw log only, is assigned zero volume. Since the origin of coordinates is transferred to the volume of tree A,

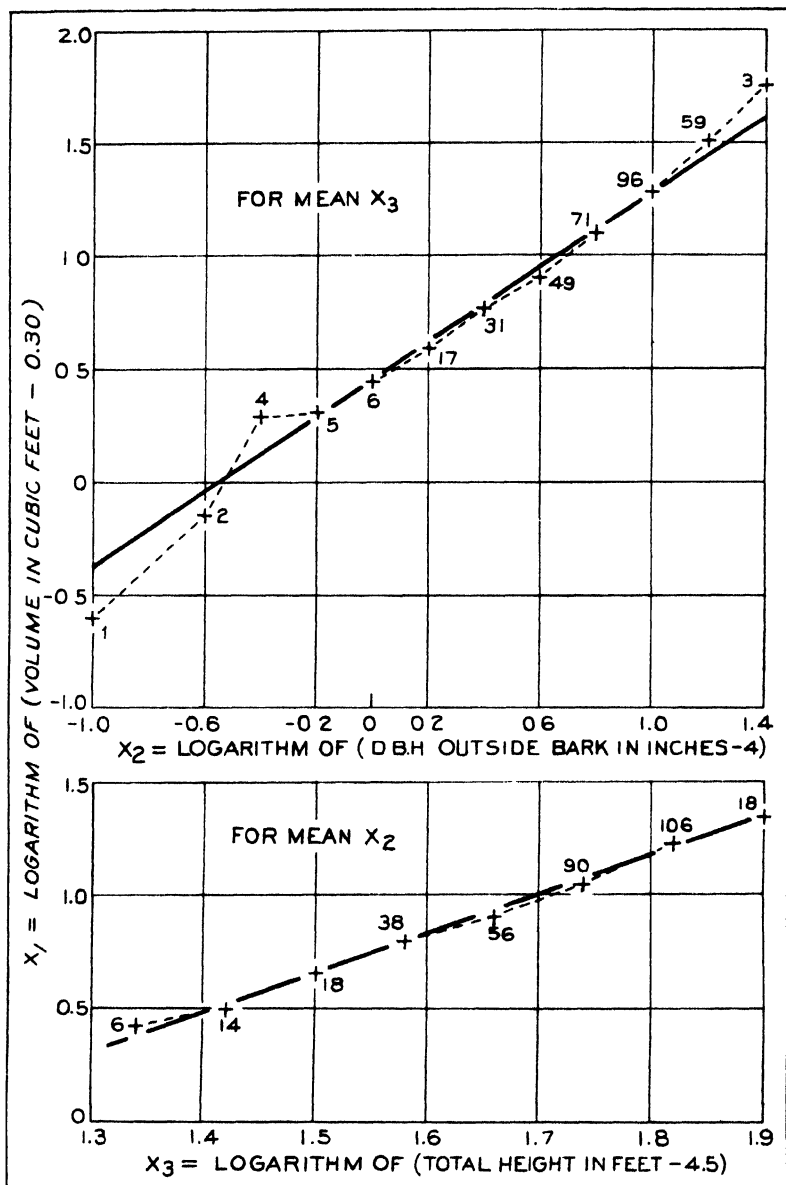


FIGURE 3 Graphic test of the linearity of a logarithmic volume equation based on measurements of 400 black walnut trees. The dependent variable is the logarithm of the cubic-foot volume to a constant top diameter less 0.30 cubic foot.

tree B must be discarded from the study, not because its independent variable, d.b.h., is below the fixed limit, but because its dependent variable, volume, is below the limit of one full saw log. Now, if obser-

vations are discarded because of restrictions placed upon the dependent variable, the regression equation is affected; for discarding the zero volumes increases the average volume of the remaining small trees, and thus lessens the regression slope of volume on diameter of these small trees. If, on the other hand, tree B is retained and zero volume assigned to it, $V-13$ becomes negative and its logarithm cannot be determined. The only alternative is to transfer the restriction from volume to the independent variable, d.b.h. inside bark. If this is done the regression equation is not affected. In the present instance this change is made by assigning the volume of the fractional saw log to trees that do not contain a full log, and then putting the origin of coordinates at 5 inches in d.b.h., at 4.5 feet in total height, and at $(\frac{3.5}{16} \times 13)$ board feet in volume. This treatment is more strictly in accordance with equation (6).

Figure 5 shows the graphic test of the linearity of the relationship of the logarithms of volume with the logarithms of diameter and height for 105 longleaf pines, which have been scaled in 16-foot saw logs by the International log rule of $\frac{1}{8}$ -inch kerf between a stump height of 1 foot and a top diameter limit of 10 inches inside bark, the logarithms of volume being expressed by the equation

$$\log (V-15.64) = 1.0422 \log (D-10) + 0.9469 \log (H-4.5) - 0.0541.$$

Figure 6 shows the same test for 275 shortleaf pines, which have been scaled in 16-foot saw logs by the Scribner log rule between a 1-foot stump and a top diameter of 8 inches inside bark, the logarithms of volume being expressed by the equation

$$\log (V-7.7) = 1.0512 \log (D-8) + 1.2609 \log (H-4.5) - 0.8023.$$

The fit of each set of data to its own equation is very good.

Attempts to substitute merchantable height for total height in volume equations of this type have so far been unsuccessful, in that the resulting logarithmic relationships have not been linear.

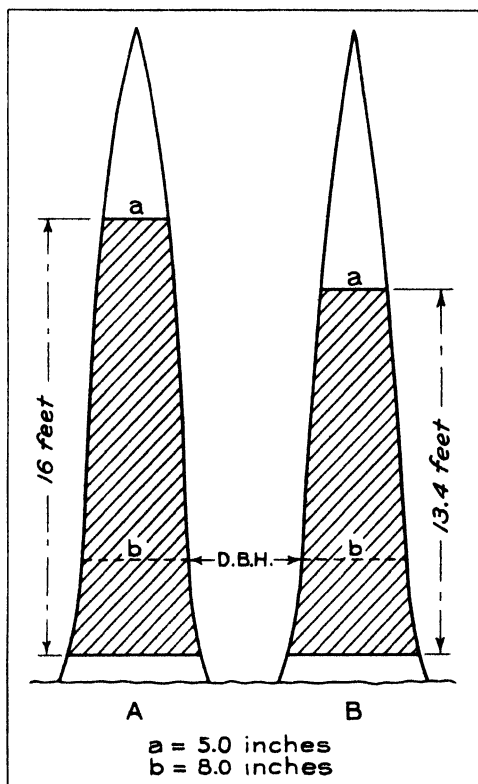


FIGURE 4. - A and B represent two trees identical in d.b.h. and in total height. Tree A contains just one full saw log of 13 board feet. Tree B contains somewhat less than a full-length saw log, and so under the usual definition of board-foot contents is assigned zero volume.

(D) BOARD-FOOT VOLUME TO A VARIABLE TOP DIAMETER INSIDE BARK IN TERMS OF D.B.H. INSIDE BARK AND MERCHANTABLE HEIGHT

Girard⁷ has found that the actual diameter limit to which saw logs are cut from the tree tends toward a constant percentage of d.b.h. for

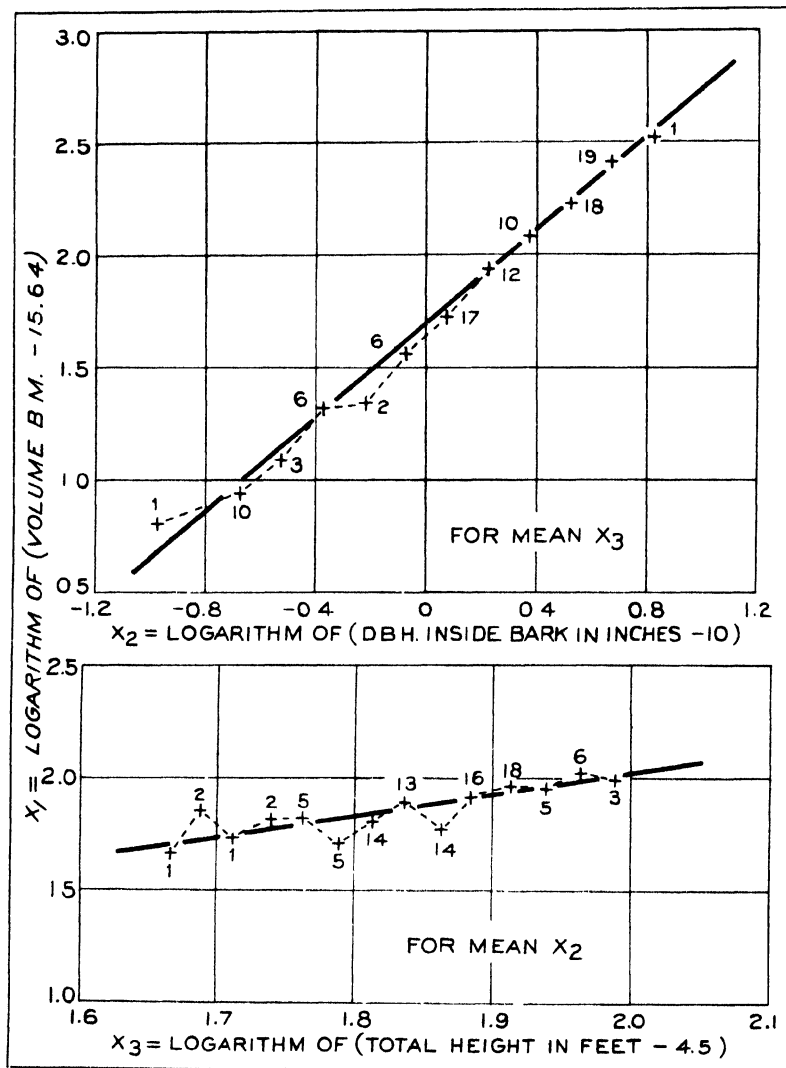


FIGURE 5.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 105 longleaf pine trees. The dependent variable is the logarithm of board-foot volume by the International log rule to a constant top diameter of 10 inches, less 15.64 board feet.

a given species. The resulting conception of board-foot volume in the tree lends itself to a logarithmic equation of the same type as equation (6), and has the advantage that the equation is linear even when the height variable is the used length rather than the total height of tree.

⁷ GIRARD, J. W. VOLUME TABLES FOR MISSISSIPPI BOTTOMLAND HARDWOODS AND SOUTHERN PINES. *Jour. Forestry* 31: 38, 1933.

We have

$$(V-v) = (D-\frac{1}{2}D)^{b_1} (H-h)^{b_2} C.$$

In this case V is volume in board feet from stump to a top diameter

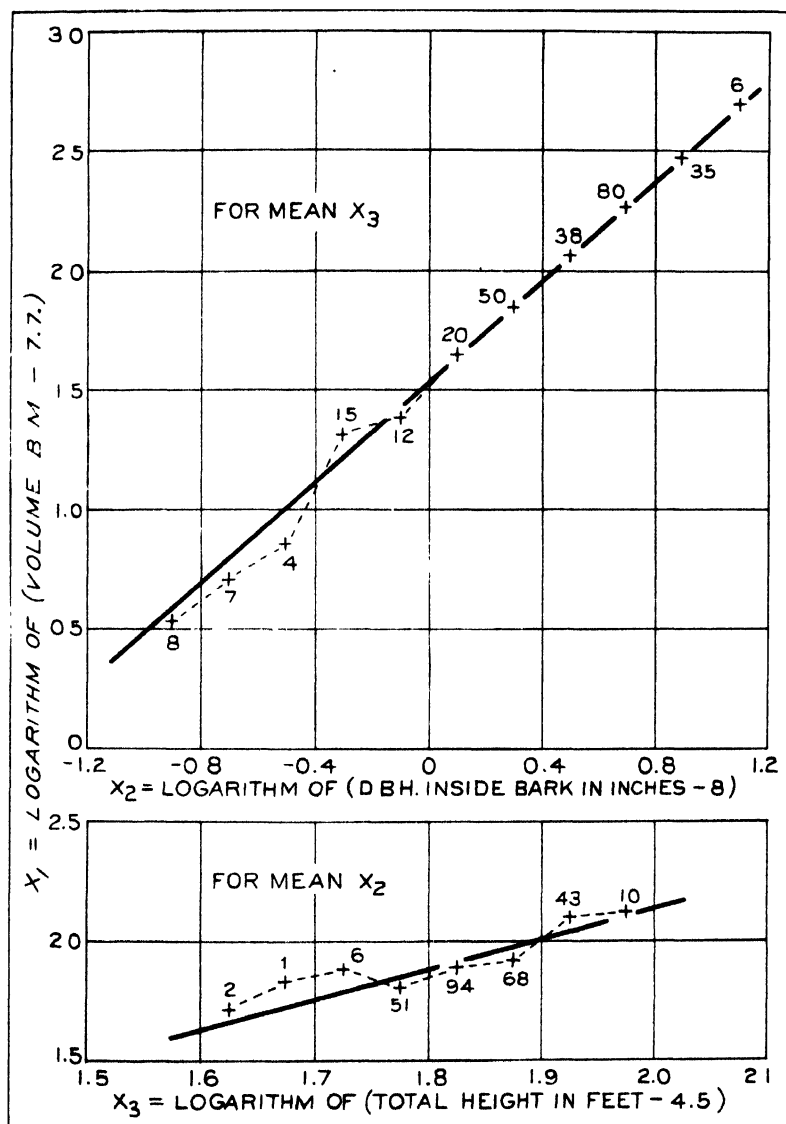


FIGURE 6.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 275 shortleaf pine trees. The dependent variable is the logarithm of board-foot volume by the Scribner log rule to a constant top diameter of 8 inches, less 7.7 board feet.

equal to $\frac{1}{2}$ d.b.h. inside bark, D is d.b.h. inside bark, H is height from stump to the point of $\frac{1}{2}D$, h is length from stump to breast height, and v is the board-foot volume of a log of h length and $\frac{1}{2}D$ diameter. The

equation may be somewhat simplified, for

$$(D - \frac{1}{2}D)^{b_1} = \frac{D^{b_1}}{2^{b_1}}$$

The logarithmic form, then, is as follows:

$$\log (V - v) = b_1 \log D + b_2 \log (H - h) + C - b_1 \log 2.$$

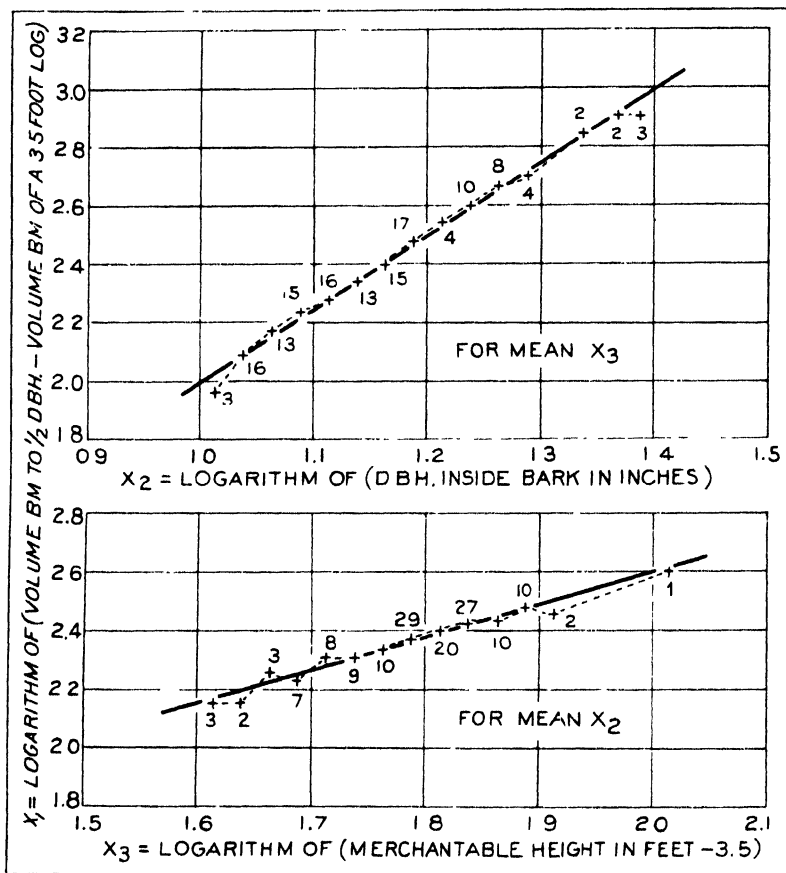


FIGURE 7.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 141 loblolly pine trees. The dependent variable is the logarithm of board-foot volume by the Scribner log rule to a top diameter equal to $\frac{1}{2}$ d.b.h. less the board-foot contents of a 3.5 foot log having a diameter equal to $\frac{1}{2}$ d.b.h.

Since $(-b_1 \log 2)$ is a constant, it is included in the C term. Figure 7 shows the graphic test of linearity of the logarithm of the board-foot contents of 141 loblolly pines, the volumes being expressed by the equation

$$\log (V - v) = 2.4995 \log D + 1.1050 \log (H - 3.5) - 2.4841.$$

The trees were scaled in 16-foot logs, by the Scribner rule, from stump height of 1 foot to a top diameter inside bark of $\frac{1}{2}$ d.b.h. It is evident that the equation is a linear one of excellent fit.

COMPARISON BETWEEN SAMPLES OF THE SAME OR OF DIFFERENT SPECIES

Comparison of the volume equations of two different species or of two samples of the same species resolves itself into a test of the significance of the difference between the average values estimated from the two equations. The error of a particular tree's actual volume (expressed in logarithms) as compared with its estimated volume (expressed in logarithms) is, of course

$$X_1 \text{ observed} - X_1 \text{ calculated}$$

X_1 being the logarithm of the dependent variable, volume. And σ_{1-23} , the standard error of estimate of a single tree typical of the entire set, is obtained from the relationship

$$\sigma_{1-23}^2 = \frac{\Sigma(X_1 \text{ observed} - X_1 \text{ calculated})^2}{N - 3}$$

in which Σ is the sum of the values enclosed within the parentheses and N is the total number of trees from which the three parameters of the equation are deducted. It is, of course, not necessary to compare every observed X_1 with its calculated value in order to compute the standard error of estimate, for

$$\sigma_{1-23}^2 = \frac{N\sigma_1^2(1 - R_{1-23}^2)}{N - 3}$$

in which R_{1-23} is the multiple correlation coefficient of X_1 on X_2 and X_3 . The standard error of estimate of the mean X_1 —i.e., M_1 —of all the trees used in the regression equation is given by

$$\sigma_{M_1}^2 = \frac{\sigma_{1-23}^2}{N} \quad (7).$$

Using equation (7) we may readily test whether, for example, the mean logarithm of cubic-foot volume of the entire stem inside bark of yellow poplar is significantly different from the corresponding mean for red gum. Taking the values of M_1 , σ_{1-23} , and N from table 2, we find that the two means with their corresponding standard errors are as follows:

For red gum: 0.9766 ± 0.00586 .

For yellow poplar: 0.9340 ± 0.00226 .

The difference between the means is 0.0426. The standard error of the difference is

$$\sigma_{a-b}^2 = \sigma_a^2 + \sigma_b^2 \quad (8)$$

in which σ_a and σ_b are the standard errors of the two independent values which we are comparing, namely, the means for the two species. From equation (8) the standard error of the difference between the means of the two species is found to be 0.0063. The difference is nearly seven times the error of the difference, and is therefore significant.

On the other hand, the higher M_1 of red gum is due not to a different regression equation but to the fact that the average dimensions of red gum, both d.b.h. and height, are greater. For by putting X_2 and X_3 equal to the red gum M_2 and M_3 (from table 2), respectively,

in the yellow poplar equation, we obtain the new mean

$$M_1 = 1.7924 (0.9039) + 1.0565(1.7858) - 2.5220 = 0.9849$$

which is 0.0083 higher than the red gum mean. Now the standard error of this estimate depends upon the average values of X_2 and X_3 used in the equation. It is the standard error of the function, i.e., of the regression equation, and is given by Miner.⁸ Its value is

$$\sigma_f^2 = \frac{\sigma^2_{1.23}}{N} \left(1 + \frac{(X_2 - M_2)^2}{\sigma_2^2(1 - r_{23}^2)} + \frac{(X_3 - M_3)^2}{\sigma_3^2(1 - r_{23}^2)} - \frac{2r_{23}(X_2 - M_2)(X_3 - M_3)}{\sigma_2\sigma_3(1 - r_{23}^2)} \right) \quad (9)$$

in which σ_f is the standard error of the function. If the average values of X_2 and X_3 of the group are equal to M_2 and M_3 upon which the regression equation is based, then $\sigma_f = \sigma_{M1}$, for the terms containing X_2 and X_3 become zero.

Applying equation (9) in order to calculate the standard error of this value—the mean logarithm of the volume which yellow poplar would have if its mean d.b.h. and height variables were identical with those of red gum—we have $(X_2 - M_2) = (0.9039 - 0.8853) = 0.0186$ and $(X_3 - M_3) = (1.7858 - 1.7699) = 0.0159$, respectively. The standard error of this new mean is

$$\sigma_f = 0.00226 \left(1 + \frac{(0.0186)^2}{(0.0557)(0.2026)} + \frac{(0.0159)^2}{(0.0237)(0.2026)} - \frac{2(0.8931)(0.0186)(0.0159)}{(0.0363)(0.2026)} \right)^{1/2} = 0.00227$$

which is so nearly the same as σ_{M1} for yellow poplar—i.e., 0.00226—because $(X_2 - M_2)$ and $(X_3 - M_3)$ are very small.

Following, then, is the comparison of the mean logarithm of volume of the two species when the mean logarithms of their d.b.h. and of their height variables are identical:

For red gum: 0.9766 ± 0.00583 .

For yellow poplar: 0.9849 ± 0.00227 .

The difference is 0.0083 ± 0.0063 , which is not significant. Therefore, if these two samples represent their respective species in a given forest, the volumes of yellow poplar may be estimated from the red gum volume table as derived from the logarithmic volume equation for red gum. If the corresponding regression coefficients of the two species were identical, such a comparison would hold throughout the joint range of the diameter-height distributions.

In table 3 are listed the regression coefficients with their standard errors. The latter are derived from the relationships

$$\sigma_{b^2_{12.3}} = \frac{\sigma^2_{12.3}}{N\sigma_2^2(1 - r_{23}^2)}$$

$$\sigma_{b^2_{13.2}} = \frac{\sigma^2_{1.23}}{N\sigma_3^2(1 - r_{23}^2)}$$

That the differences between the regression coefficients cannot be ascribed to differences in the characteristics of the species is apparent.

⁸ MINER, J. R. THE STANDARD ERROR OF A MULTIPLE REGRESSION EQUATION. *Ann. Math. Statist.* 2:320-323. 1931.

It follows that if there is a region of the diameter-height distribution for which the volume estimates of the two species differ in a significant degree, this region must be at the extremities of the joint distribution. Upon comparing the calculated values of X_1 for several combinations of X_2 and X_3 , we find that significant difference occurs only in the trees of small diameter, particularly those of lesser height. At $X_2 = 0$ and $X_3 = 1$, for instance, the estimated X_1 for red gum is -1.52 ± 0.021 while that for yellow poplar is -1.47 ± 0.012 . The difference between these estimates is 0.05 ± 0.024 , which is greater than twice its standard error. Except for very small trees, then, either equation should afford a satisfactory volume estimate for either species.

TABLE 3.— *Comparison of the logarithmic regression coefficients of yellow poplar with the corresponding coefficients of red gum*

Species		
Yellow poplar	1 7924 $\pm$ 0.02123	1 0565 $\pm$ 0.03262
Red gum	1 7470 $\pm$ 0.06494	1 1644 $\pm$ 0.08801

EXPRESSION OF LOGARITHMIC EQUATIONS IN ALINEMENT CHART FORM

The logarithmic forms of the volume equations which have been discussed are equations of the first degree in three variables. Hence they may be expressed in alinement-chart form with three straight, parallel axes upon which the logarithms of the variables are graduated in arithmetic scale. A method of constructing regression equation alinement charts in general is discussed by Bruce and Reineke.⁹

Figure 8 is an alinement chart of the board-foot volume equation for shortleaf pine to a top diameter inside bark of 8 inches by the Scribner rule. The left-hand sides of the outer axes are graduated in terms of the functions of d.b.h. [$-1.0512 \log (D-8)$] and of total height plus the constant term [$-1.2609 \log (H-4.5) - 0.8023$], respectively; the sum of these gives the function of volume [$-\log (V-7.7)$], and this is graduated on the left-hand side of the middle axis. A straightedge connecting any two points on the outer axes intersects the middle axis at their sum. The graduations on the right-hand side of the axes are D , H , and V corresponding to their respective functions. If the graduations of the functions are erased the volume alinement chart is in its usual form.

SUMMARY

Timber-tree volume equations of the power or logarithmic type in terms of diameter and height are deduced from known volume equations of geometrical solids on the theory that the volume of the tree stem, unlike that of the geometrical solid, may vary not directly as height and as the square of diameter, but as other powers of these dimensions in both cubic feet and board feet. The theory is tested by transforming the power equation into a linear one in logarithms and calculating the logarithmic regression equations for available

⁹ BRUCE, D., and REINEKE, L. H. CORRELATION ALINEMENT CHARTS IN FOREST RESEARCH. U.S. Dept. Agr. Tech. Bul. 210, 88 p., illus. 1931.

tree data. Comparison of the actual tree volumes with those estimated from the equations confirms the hypothesis beyond practical doubt.

$$V = \frac{1}{6343} (D-8)^{1.0512} (H-4.5)^{1.2609} + 7.7$$

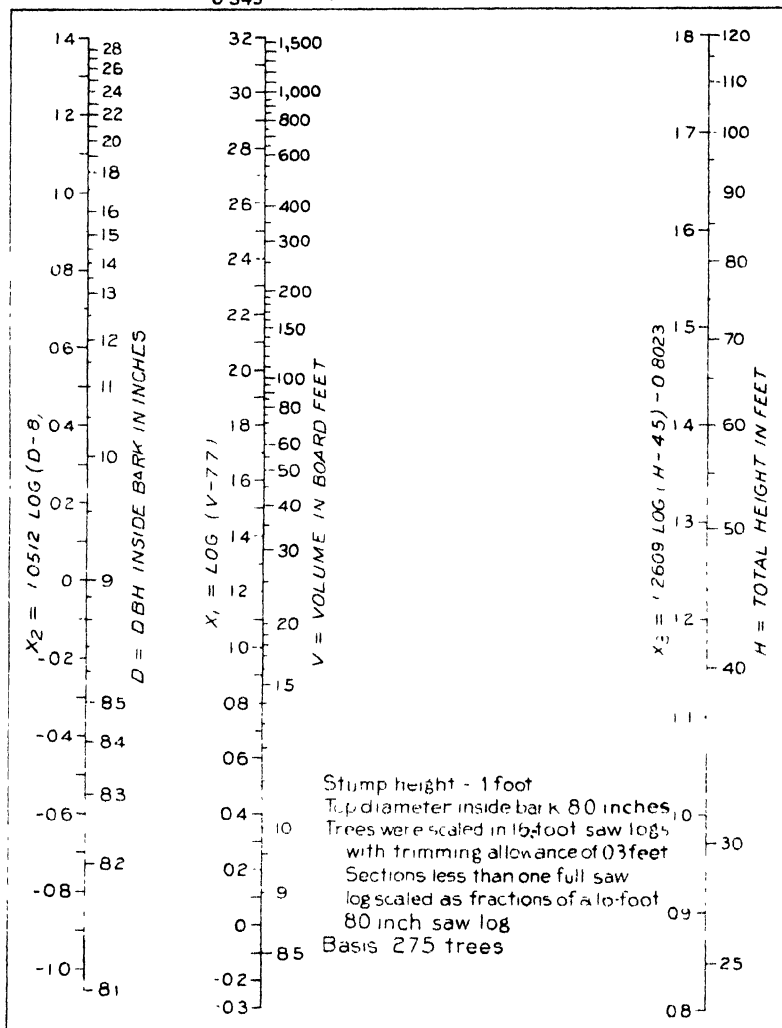


FIGURE 8 -Volume alignment chart constructed from a volume equation for shortleaf pine

The usefulness of the standard error of the regression equations in testing the significance of the difference between two sets of data, and the expression of the equations in volume alignment chart form, are illustrated.

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ESTIMATED DATA ON THE ENERGY, GASEOUS, AND WATER METABOLISM OF POULTRY FOR USE IN PLANNING THE VENTILATION OF POULTRY HOUSES

By H. H. MITCHELL, *chief in animal nutrition, Department of Animal Husbandry, Illinois Agricultural Experiment Station*, and M. A. R. KELLEY, *agricultural engineer, Division of Structures, Bureau of Agricultural Engineering, United States Department of Agriculture*¹

INTRODUCTION

There seems to be a dearth of reliable estimates of the production of heat, carbon dioxide, and water by the different classes of poultry, for the use of agricultural engineers in the designing of ventilating systems for poultry houses. This paper presents a series of estimates of the nature required, covering the entire growing period of a large (Plymouth Rock) and a small (Leghorn) breed of chickens, of the Bronze turkey, of the Pekin duck, and of Embden and Toulouse geese.

In the Divisions of Animal Nutrition and Poultry Husbandry of the University of Illinois, an intensive study of the growth, metabolism, and nutritive requirements of chickens has been under way for several years, and a similar project on the Bronze turkey has been initiated. The information thus collected and in part published (22, 23, 24, 25, 26, 27)² was used as the basis of most of the estimates herein reported. Direct application of this information has been made to the two breeds of chickens included in the series of estimations made, and indirect application has been made to turkeys, ducks, and geese, on the basis of a number of assumptions, very few of which can be defended except by the logic of analogy. The excuse for proceeding on this basis is (24) that, on the few specific phases of metabolism for which information on a number of kinds of poultry is available, no distinct differences have been noted, and, moreover, (25) no other basis of estimation appeared at hand.

METHODS OF ESTIMATION

The general procedure followed in making the estimations has been to factor out and to assess as accurately as possible all of the items contributing to the energy requirements and expenditures of the birds and to their requirements for water, and then to integrate these factors for birds of different weights, to get the values desired. In pursuing this course a number of assumptions have been made. These, as well as the integrations leading to the information desired, are here described.

The basal metabolism of all classes of poultry per square meter of body surface is assumed to be the same at equivalent ages, and to be equal to the values reported by Mitchell, Card, and Haines (23) in a comprehensive investigation of the metabolism of chickens.

¹ Received for publication June 14, 1933; issued January, 1934.

² Reference is made by number (italic) to Literature Cited, p. 747.

The basal metabolism of mature chickens averages about 800 cal. per square meter for males and 750 cal. per square meter for females. Herzog (13) has reported an average of 874 cal. for six pigeons, and values of 907, 891, and 1,093 cal. for a gander, a goose, and a duck, respectively. These values, and especially the last, probably include some heating effect of food, since the pigeons were fasted only for 20 to 24 hours, the geese for 12 to 16 hours, and the duck for 8 to 12 hours. Workers at this laboratory have found that in determining basal metabolism a fasting period of 48 hours is required for chickens in order that there may be no residual effect of the last feeding; Benedict and Riddle (3) found that for pigeons a fast of 24 hours is required for the attainment of fasting respiratory quotients. Riddle, Nussmann, and Benedict (30) measured the basal metabolism of the common pigeon from an age of 21 days to an age of 439 days, and observed essentially the same relations as those obtained for chickens. Finally, Hári obtained measurements of 682 to 1,038 cal. per square meter for the basal metabolism of geese (10) and 735 to 935 cal. for ducks (11).

These citations justify the essential accuracy of the assumption that the basal metabolism of all poultry is much the same at equivalent ages, i.e., ages at which the same fraction of the mature weight has been attained. The disagreement between the values cited, aside from technical errors, may be largely due to the use of formulas for estimating surface area from weight which possess different degrees of accuracy.

The extra heat due to voluntary activity is assumed to approximate one half the basal heat. The justification of this relation for chickens is discussed fully by Mitchell, Card, and Hamilton (25, p. 133). The absence of any information whatever on this point for other classes of poultry necessitated the general use of this figure throughout. Specific investigation with turkeys, ducks, and geese will be required before the magnitude of the error incurred by proceeding on this assumption can be determined.

Although specific information was found with reference to the growth rates of the different classes of poultry, the energy content of the gains had to be computed from the data available for chickens. The data for White Plymouth Rocks were taken directly from Illinois Bulletin 276 (24), and those for White Leghorns from Illinois Bulletin 367 (25).

The chemical composition of turkeys, ducks, and geese has been but little studied. From such analyses as were available, however, (1, 12, 18, 19) it appears that turkeys contain about as much fat as chickens, ducks are considerably fatter, and geese even more so. In making the computations summarized in the tables it was assumed that, at equivalent ages, turkeys put on gains in weight possessing the same chemical composition as the gains of chickens, but that ducks put on gains containing half again as much fat, and geese put on gains containing twice as much fat.

The total net energy requirement is, of course, equal to the sum of the energy expended in basal metabolism, the energy expended in muscular activity, and the energy equivalent of the gain in weight.

The net energy requirement is stated also in terms of dry matter required, the dry matter being assumed to have the net energy value of wheat for chickens (18, 19), i.e., 282 cal. per 100 g.

The heating effect of the feed is taken at 68 cal.³ per 100 g of dry matter, this being the heat increment of wheat for chickens (18, 19).

The total heat production is the sum of the basal heat, plus the heat liberated in muscular activity, plus the heat increment due to feed.

In estimating the output of carbon dioxide from the total heat production, an average respiratory quotient of 1 was assumed for the day. The justification for this assumption is found in the respiration experiments of Mitchell and Haines (27) on chickens and the measurements on their control birds by Baldwin, Nelson, and McDonald (2) and by Caridroit (5). On this assumption, 1 l of CO₂ is produced for each 5.047 cal. of heat.

The estimations of water metabolism involved all of the following factors: (a) Water-vapor elimination, (b) excretion of liquid water in the excrement, (c) production of metabolic water in the body, and (d) storage of water in the tissues during growth. Having estimated these factors according to what seemed to be reasonable procedures, the water requirement was computed as being equal to $a + b + d - c$.

From Hári's respiration experiments on geese (10), it appears that at 16° C. an average of 20 percent of the heat output is lost in the vaporization of water, and at 28° an average of 40 percent is thus lost. At a body temperature of approximately 41° all the heat produced must be lost as vaporized water. These meager figures on geese, as well as data on other animals that might be cited, indicate clearly a rapidly increasing percentage of heat lost as vaporized water as the environmental temperature increases. This relation appears to be a logarithmic one, and Hári's data are well described by the following equation, the constants of which were determined by the writers.

$$W = 7.14 e^{0.06438t}$$

in which W is the percentage of the heat produced that is dissipated as the heat of vaporization of water, and t is the environmental temperature expressed in degrees centigrade. From this equation the following values were computed:

°C	°F.	W
0	(32)	7.1
4.5	(40)	9.5
10	(50)	13.6
15.5	(60)	19.4
21.0	(70)	27.6
26.5	(80)	39.3
32.2	(90)	56.7
37.7	(100)	81.1
41	(106)	100

In the estimations made on all classes of poultry it was assumed that 40 percent of the heat production was represented in the vaporized water and that this condition, in normally feathered birds, obtained at 28° C. (82° F.). Hence the vaporized water at this temperature was estimated from the total heat production by multiplying by 0.4 and dividing by 0.58, the heat of vaporization of 1 g of water at 30° C. (86° F.), a figure approximating average skin

³ No allowance for a variable heating effect of feed as the level of feeding is varied can be made in the absence of information on this point. However, it has been found (27) that the heating effects of 50 and 75 g of corn for chickens are much the same per 100 g of corn fed.

temperature. For other environmental temperatures, the vaporized water may be computed by means of the tabulation given above. The maximum vaporized water, obtained at temperatures equal to or exceeding body temperature is, of course, computed from the total heat production merely by dividing by 0.58. It should be pointed out here that heat lost in vaporized water is not available for heating the surroundings unless the water vaporized is condensed.

The elimination of liquid water in the excreta was computed from the dry matter consumed on the assumption that the dry matter was 20 percent indigestible and that the mixed excreta contained 27 percent of dry matter. The latter value was obtained from a number of published digestion experiments on chickens in which the mixed excreta were analyzed for moisture. It is recognized, of course, that this value is subject to variation.

The stored water was computed from the daily gains in each species and the composition of gains in chickens as determined at the Illinois Station.

The metabolic water, that is, the water produced in the body by the oxidation of the food nutrients, was computed on the basis of the following commonly used factors:

100 g of protein yield 41 g of water.

100 g of fat yield 107 g of water.

100 g of carbohydrate yield 60 g of water.

Considering the fact (1) that the metabolic mixture from which the metabolic water originates is equal to the mixture of nutrients consumed minus the mixture of nutrients stored in the body, and (2) that the nutrient mixture in wheat, in terms of which the dry-matter requirements are expressed, is mainly carbohydrate in character, it was decided to compute the metabolic water in all cases as 48 percent of the dry-matter requirement. It is realized that the metabolic water might have been estimated by a more refined method that would have taken into account the varying rates of storage of nutrients at different stages of growth, but the greater accuracy thus obtained would have been secured at the expense of much greater labor, while the error incurred by the simpler method is a relatively small item in the water values desired, and is merely one of a large number of errors to which the estimation of each of the factors in the problem are undoubtedly subject.

For Bronze turkeys, the growth data of Funk and Margolf (8) have been used. The growth obtained in these experiments is considerably more rapid than that reported by Mussehl (28). In fact, it does not show the slowing that might have been expected at the greater ages, and no success was had in fitting to the data the mathematical expression of the law of diminishing returns that has been used so successfully by Brody in describing the growth of animals for all but the first small fraction of the growth period. The adult weight of the male Bronze turkey is taken to be 36 pounds and that of the female 20 pounds.

For Pekin ducks, the data of Horton and the mathematical analysis of them made by Titus (36) have been followed. These data were obtained on a flock in which the sexes had not been separated. The growth rate in this flock was somewhat greater than that later reported by Horton (15) and also than that reported by Lehmann (21), by

Spyra (33), or by Tallent (34). Some unpublished data obtained through the courtesy of Professor Carrick, of Purdue University, show that more rapid growth of Pekin ducks is possible for the early weeks of life, but these data show a much less sustained growth than those of Horton. The adult weight of the male Pekin duck is said to be 9 pounds and that of the female 8 pounds. The large majority of all ducks raised in this country are of the Pekin breed.

The only comprehensive study of the growth of geese that has been found in the literature is that of Cushman (6, pp. 327-358) obtained at the Rhode Island Agricultural Experiment Station and published in 1895. Since the Embden and Toulouse are the breeds of geese most commonly raised in this country and also the breeds used to the greatest extent in the Rhode Island experiments, no other breeds are considered in this paper. Although the adult weights of Toulouse geese are greater than those of Embden, i.e., for the males, 26 pounds as compared with 20 pounds, and for the females, 20 pounds as compared with 18 pounds, the rates of growth as observed by Cushman are quite similar up to a body weight of 11 pounds. Hence the growth of both breeds has been calculated by the same equations obtained from the combined data of Cushman. Up to a weight of 2 pounds the growth is well described by the equation

$$W = 98.63 e^{0.0718t}$$

and from this weight to a weight of 11 pounds by the equation

$$W = 9000 - 9609 e^{-0.0545t}$$

In both of these equations W is the body weight in grams at age t , expressed in weeks from hatching. That an error is incurred in extending this equation to both breeds beyond a body weight of 11 pounds is, of course, apparent. However, neither for geese nor for turkeys and ducks are the estimates at adult weights affected in the slightest by any errors inherent in the method used for the estimation of growth or of growth requirements.

In determining the basal metabolism of birds of given weights, the surface areas were computed by the use of the following formulas:

Chickens:

$$S_{\text{sq. cms.}} = 8.19 W^{0.705} \text{ gms}$$

Turkeys:

$$S_{\text{sq. cms.}} = 13.16 W^{0.667} \text{ gms}$$

Ducks:

$$S_{\text{sq. cms.}} = 9.02 W^{0.705} \text{ gms}$$

Geese:

$$S_{\text{sq. cms.}} = 10.45 W^{0.667} \text{ gms.}$$

The formula for chickens was obtained for the "Leghorn" breed (22); that for turkeys was obtained from unpublished data on the surface area of Bronze turkeys as determined by the mold method; that for ducks represents a more or less arbitrary raising of the constant in the formula for chickens in accordance with the higher Meeh constant commonly used for ducks and geese; and that for geese is the Voit formula for this species.

□ □ □

DISCUSSION OF ESTIMATED DATA

The final computations are given in tables 1 and 2. It will be noted that although the ages corresponding to the given weights are estimated for the different sexes (except for ducks and geese, for which

the data are not available), no distinction between the sexes is made with respect to the other values in the tables. For chickens and turkeys the computations were carried out in full for the two sexes, but it was then observed that the values for the different sexes at any given weight did not differ greatly, and in the large majority of cases were very nearly identical. This is because no considerable sex differences had been assumed throughout the calculations except with respect to the rates of growth and the composition of gains in weight. The faster rate of growth of the male is almost exactly compensated for, so far as the energy requirements for growth are concerned, by the higher fat content of the gains in weight put on by the female.

TABLE 1—Daily food requirements, heat production, and water used and excreted by chickens and turkeys of different weights

WHITE LEGHORN CHICKENS

Body weight (pounds)	Age		Total net energy required	Dry matter re- quired ^a	Total heat pro- duced	CO ₂ pro- duced	Water re- quired at 28° C	Water vapo- rized at 28° C	'Total water ex- creted at 28° C.	Maxi- mum water vaporized at 41° C.
	Males	Fe- males								
	Weeks	Weeks	Calories	Grams	Calories	Liters	Grams	Grams	Grams	Grams
0 077	0	0	13	5	10	2	14	7	11	17
0 5	4.0	4	80	28	82	16	72	56	77	141
1 0	6.9	8 2	104	37	110	22	99	76	103	190
1 5	9 6	11 5	124	44	132	26	117	91	124	228
2	12 5	14 8	143	51	155	31	134	107	144	267
3	18 2	22 4	173	61	194	38	161	134	179	334
4	25 4	38 3	204	72	235	47	188	162	216	405
5 ^b	33 0	- -	235	83	281	56	223	194	255	484

WHITE PLYMOUTH ROCK CHICKENS

0.077	0	0	13	5	10	2	14	7	11	17
0.5	4.6	4.6	79	28	82	16	69	56	77	141
1	6.6	6.7	108	38	110	22	96	76	104	190
1.5	8.7	8.9	137	49	135	27	118	93	128	233
2	9.7	10.7	160	57	159	32	137	110	152	276
3	12.4	14.9	201	71	200	40	168	138	191	345
4	15.7	19.4	233	82	248	47	195	164	224	410
5	19.3	25.4	258	92	280	55	224	193	261	482
6	23.3		259	92	310	62	241	214	282	535
7 ^b			285	101	354	70	270	244	319	610

BRONZE TURKEYS

0.115	0	0	24	8.5	20	4	22	14	20	34
0.5	3	3+	95	34	115	23	98	79	104	198
1	5	5+	150	53	170	34	146	117	156	293
2	7	8	221	79	239	48	208	165	223	412
3	9	10	275	98	287	57	265	198	271	495
4	10+	11	322	114	327	65	304	226	310	564
5	11+	12+	364	129	365	72	337	252	347	629
7	13	15	441	157	437	87	391	301	417	754
10	16	19	553	196	553	110	480	381	526	954
15	20		706	251	720	143	607	406	682	1,241
20	25		840	298	890	176	743	614	835	1,534
20		52	645	229	801	159	611	552	721	1,381
36	104		1,017	361	1,262	250	963	870	1,137	2,176

^a These values relate to dry matter with the net energy value of wheat, i.e., 282 cal. per 100 g.

^b Male

TABLE 2.—Daily food requirements, heat production, and water used and excreted by ducks and geese of different weights

PEKIN DUCKS

Body weight (pounds)	Age	Total net energy required	Dry matter required ^a	Total heat produced	CO ₂ produced	Water required at 28° C.	Water vaporized at 28° C.	Total water excreted at 28° C.	Maximum water vaporized at 41° C.
	Weeks	Calories	Grams	Calories	Liters	Grams	Grams	Grams	Grams
0.125	0	23	8	18	4	18	12	18	31
0.5	2 0	110	39	97	19	96	67	96	167
1	3 2	158	56	135	27	128	93	134	233
1.5	4 1	205	73	166	33	159	114	168	286
2	4 8	255	90	196	39	187	135	202	338
3	6 2	328	116	252	50	232	174	260	434
4	7 5	382	135	300	59	266	207	307	517
5	8 9	401	142	342	68	290	236	341	590
6	11 1	402	143	375	74	307	250	365	647
7	13 8	394	140	404	80	319	279	383	697
8		328	116	407	81	311	281	367	702
9		381	135	473	94	361	326	426	816

TOULOUSE AND EMBDEN GESE

0.215	0	31	11	28	6	26	19	27	48
0.5	1 7	115	41	113	22	81	78	86	195
1	3 1	179	63	154	30	145	106	153	266
1.5	3 8	233	83	182	36	185	126	192	314
2	4 4	267	95	205	41	214	141	218	353
3	4 6	313	111	242	48	244	167	258	417
4	5 8	343	122	270	53	254	186	275	466
5	6 9	370	131	308	61	273	212	290	531
6	8 2	390	138	339	67	296	234	329	584
8	11 1	430	152	392	78	332	270	380	676
10	14 5	463	164	446	88	370	308	429	769
12	18 7	488	173	493	98	400	340	468	850
15	27 5	514	182	561	111	444	387	522	967
18	45 4	526	187	617	122	474	426	561	1,064
20		529	188	654	130	496	451	586	1,128
26		619	230	805	160	615	555	725	1,388

^a These values relate to dry matter with the net energy value of wheat, i.e., 282 cal. per 100 g.^b Female^c Male

It is important to check such calculations as those given in tables 1 and 2, insofar as possible, from published information on the food intake or water intake, for example, of poultry on feeding experiments. When such comparisons are made of the dry-matter requirements computed for birds of definite weights and the actual amounts of feed consumed by birds of equal or similar weight in actual experiments, the latter values will in all cases be the larger for a number of reasons. In feeding experiments there is an inevitable scattering and wastage of food which may amount to as much as 5 or 10 percent. The food weighed out to the birds is not moisture-free, but probably contains as much as 10 to 15 percent of water. Mixed poultry rations may have a lower net energy value than wheat. When proper consideration is given to these points of difference, the values for the estimated dry-matter requirements of chickens, turkeys, and ducks do not seem to be unreasonably low, particularly when it is remembered that they have been computed on the assumption of a degree of activity which may be too low for birds not closely confined. Such comparisons may be made from the food-intake figures for chickens given by Jull (16) and Jull and Titus (17), those for turkeys given by Funk and Margolf (8), and those for ducks given by Titus (36) and Lehmann (21). In Lehmann's experiments the dry-matter intakes

are given for ducks at different stages of growth. Since this affords the most exact comparison of any available, the average values in these experiments for Lehmann's groups IA, IB, and IC, totaling 84 birds, are given (table 3).

TABLE 3.—*Dry-matter intake of ducks at different stages of growth in Lehmann's experiments*

Age in weeks	Average body weight	Dry matter consumed daily	Age in weeks	Average body weight	Dry matter consumed daily
	<i>Grams</i>	<i>Grams</i>		<i>Grams</i>	<i>Grams</i>
0 - - - -	125	- - -	5. - - -	1,434	144
1. - - - -	200	32	6. - - -	1,686	153
2. - - - -	539	74	7. - - -	1,897	157
3. - - - -	932	120	8. - - -	1,967	158
4. - - - -	1,207	125			

These ducks, growing at a somewhat more rapid rate than that assumed in the construction of table 2, consumed dry matter in very nearly the same amounts as the estimated requirements.

No published information could be found on the water requirements of poultry and only one reference to observed water consumption (7), which related to mature White Leghorn hens while laying eggs. However, through the kindness of Dr. L. E. Card and Dr. H. J. Sloan, of the Division of Poultry Husbandry, records were obtained of the food and water consumption of a number of pens of young chicks on different rations, as well as of laying hens. The significant value in this connection was considered to be the ratio of the weight of water drunk to the weight of dry food consumed. For 10 pens of chicks the average ratio for the first 10 to 12 weeks of life was 2.17 g of water to 1 g of feed. If it be assumed that the wastage of water was no greater than the wastage of feed, and that the feed contained 10 percent of moisture, then the average ratio of water to dry matter would be 2.52 to 1.

In one pen of Rhode Island Red hens (laying) containing 45 to 47 birds per pen, the ratios of water to feed consumed in 4 successive weeks were 1.70, 1.83, 1.42, and 1.42, the average being 1.59. In one pen of White Leghorn hens, containing 41 to 45 birds per pen, the ratios for successive weeks were 2.01, 1.76, 1.41, and 1.35, the average being 1.62. On the same assumptions as before, the average ratio of water to dry matter consumed becomes 1.89 to 1. The difference between the two sets of data—i.e., those for chicks and laying hens—may have been due in part to the fact that the former were collected mainly in the warm months of June, July, and August, while the latter were collected from April 7 to May 4, inclusive.

If the ratios of the estimated amounts of water to dry matter required are computed from the data in tables 1 and 2, they will be found to vary from 2.0 to 1 to 2.7 to 1 and to average close to 2.4 to 1. These results are in good agreement with the chick data cited above, and apply to fairly warm weather. For growing mice, Bing and Mendel (4) have reported an average ratio of 1.3 g of water consumed to 1 g of food. The senior writer has obtained values for poultry approximating this one, if the water requirement is assumed to equal 800 cc per square meter of body surface, as Richter and

Brailey (29) have found with growing rats. However, the weight of evidence seems to establish the fact that the water requirements of poultry are higher than these estimates indicate, possibly because of the higher body temperature.

In using the water data in tables 1 and 2, it should always be remembered that these data are subject to the greatest variation of all. They are dependent particularly upon the environmental temperature, as pointed out above, and also, in an undetermined manner, upon the environmental humidity, a high humidity tending to depress the vaporization of water from the body. Probably also changes in the feather covering have a considerable effect on the vaporization of water, and the character of the ration fed affects the amount of water consumed. The content of the diet in protein and in salts that are excreted by the kidney must also modify the amount of water consumed, the relation being a direct one.

If the environmental temperature sinks to very low levels, the heat production of poultry will increase beyond the values given in the tables, in order to maintain normal body temperatures. The increase will be secured either by an increased muscular activity or by an increased muscle tonus, or by both; or possibly even by an increased consumption of feed. Some rough estimates may be made of the environmental temperature at which an increase in heat production must occur. For these calculations a critical temperature for the fasting bird of 62° will be used. This temperature was determined by Mitchell and Haines (26) in experiments with a considerable number of chickens. Terroine and Trautmann (35), however, have reported higher critical temperatures for a hen and a goose. For a bird consuming food and moving about normally, there is an excess production of heat above the basal which, at temperatures below 62° is available to maintain body temperature. This excess heat consists of (1) the heat liberated by muscular activity, assumed in these calculations to equal one half of the basal heat, and (2) the heat increment produced by the feed, assumed to equal 68 cal. per 100 g of dry matter consumed. For the different classes of poultry and for the different body weights, the extra heat available for this purpose is given in table 4. Table 4 gives for each species an estimate of the heat required to maintain body temperature, in excess of that furnished by the basal metabolism, for each degree of fall in environmental temperature below 62° . This estimate is based on the validity of Newton's law of cooling bodies as applied to the bird under basal conditions at its critical temperature (26, p. 556), and is equal to the basal heat production of the bird divided by 44, the difference between the body temperature, 106° , and the critical environmental temperature in the fasting condition, 62° . Table 4 also gives for each species an estimate of the critical temperature of the active bird consuming its required food.⁴ This temperature would be considerably higher for the bird while asleep or otherwise inactive.

⁴ For instance, for a 2-pound Pekin duck the critical temperature = $62 - (106 \div 2.0) = 9$.

TABLE 4.—Calculation of critical temperatures for different species of poultry of varying weights

Body weight (pounds)	White Leghorn chickens			White Plymouth Rock chickens			Bronze turkeys			Pekin ducks			Geese		
	Extra heat available	Heat required per ° F. drop below 62° F.	Critical temper- ature	Extra heat available	Heat required per ° F. drop below 62° F.	Critical temper- ature	Extra heat available	Heat required per ° F. drop below 62° F.	Critical temper- ature	Extra heat available	Heat required per ° F. drop below 62° F.	Critical temper- ature	Extra heat available	Heat required per ° F. drop below 62° F.	Critical temper- ature
0.5	Cals 42	1 02	21	Cals 42	1 02	21	Cals 46	1 11	21	Cals 52	1 14	16	Cals 44	Cals 89	° F. 13
1	51	1 2	19	52	1 2	19	86	2 1	21	68	1 4	13	80	1 7	15
1.5	64	1 5	19	67	1 5	17	88	1 7	10	88	1 7	10	101	2 0	12
2	75	1 8	20	79	1 8	18	115	2 8	21	106	2 0	9	117	2 2	9
3	94	2 3	21	99	2 3	19	136	3 6	18	136	3 6	10	138	2 7	11
4	111	2 8	22	118	2 8	20	158	3 6	18	161	3 2	12	142	2 8	11
5	131	3 4	23	135	3 4	22	175	4 0	18	179	3 7	14	154	3 2	14
6				146	3 8	24				190	4 2	17	171	3 6	14
7				164	4 3	24	216	5 0	19	203	4 7	19			
8							188	5 0	0	219	5 8	8	198	4 3	16
9															
10							274	6 4	19				223	5 0	17
12													242	5 7	20
15							353	8 3	19				269	6 6	21
18													280	7 5	23
20							402	10 1	22				303	8 0	24
26													372	9 8	24
36							584	15 4	24						

All such estimates as those above given are subject to great variation. The critical temperature will be higher (1) the less fat the bird contains, (2) the less active it is, (3) the less complete its coat of feathers, (4) the more humid its surroundings, (5) the less food it consumes and the longer the time from the peak of its digestion, (6) the larger the volume and the lower the temperature of the water drunk, and so forth.

No estimates have been made for the female bird while laying eggs. Under this condition, the net energy requirements are increased, as are also the consumption of dry food and water and the elimination of heat, carbon dioxide, and moisture. Thus, if a pullet is producing one egg a day of average size (58 g), its net energy requirements will be increased by 95 cal. (25), its dry-matter (wheat) requirements by 34 g ($95 \div 2.82$), its heat production by 23 cal. (0.34×68), and its carbon dioxide production by 4.5 l ($23 \div 5.047$), etc. The water requirements will be increased in proportion to the increase in dry feed ($34 \text{ g} \times 2.2 = 75 \text{ g}$) and to the water stored in the egg ($58 \text{ g} \times 0.75 = 43 \text{ g}$), making a total increase of 118 g. Estimates for ducks and geese may be computed from the analyses of duck and goose eggs made by Hepburn and Katz (12). These estimates are given in table 5. The average egg production of a duck, according to Lamon and Slocum (20), ranges from 80 to 120 per year; that of a goose ranges from 12 to 36.

TABLE 5.—Chemical composition of duck and goose eggs

Kind of egg	Average weight	Shell	Chemical composition of edible portion			Total constituents in average egg			
			Water	Fat	Protein	Water	Fat	Protein	Energy
Duck	Grams 91	Percent 10.6	Percent 68.5	Percent 14.4	Percent 13.6	Grams 56	Grams 11.7	Grams 11.1	Calories 174
Goose	205	12.7	71.2	12.2	14.1	127	21.8	25.2	351

* Taken from Horton (15a).

† Taken from Lamon and Slocum (20).

DISCUSSION OF THE DATA FROM AN ENGINEERING STANDPOINT

The agricultural engineer has long needed some source to which he might turn for such essential metabolism data relating to poultry as could be used in designing heating or ventilating systems for poultry houses. The data here recorded give the basal and normal heat production of various breeds of chickens, ducks, geese, and turkeys from the time of hatching to approximate maturity. Hence the data are applicable under a wide range of conditions. A single example of their application may be of value.

Assume an ordinary shed-roof house, 20 by 30 feet, housing 200 White Plymouth Rock hens averaging 4 pounds in weight. The floor area is 600 square feet and the volume approximately 4,200 cubic feet. Referring to table 1 we find that a 4-pound White Plymouth Rock bird will consume 82 g of dry matter, produce 238 calories of heat and 47 liters of carbon dioxide, and vaporize 164 g of water daily. If these data are converted into terms commonly used by engineers, 200 hens will produce hourly 7,866 B.t.u. and vaporize 3 pounds of water per hour, making 21,000 grains of moisture to be removed by ventilation. However, these data are given for an environmental temperature of 82° F. At this temperature an ordinary poultry house would be wide open. If a house temperature of 50° F. is assumed,⁵ the moisture production would be much less. The tabulation given on page 737 shows that at this temperature 14 percent of the heat produced is dissipated as water vapor, which, according to the method of estimation herein reported, would make 57 g of water produced daily by such a bird. $(238 \times 0.14) \div 0.58 = 57$; $57 \times 200 = 11,400$ g daily, or 7,315 grains or 1.045 pounds hourly. This represents the amount of water to be removed hourly from the henhouse.

The temperature maintained within the house depends upon the balance between the heat produced and the heat saved. The data given in tables 1 and 2 are for temperatures of 28° and 41° C. (82.4° and 105.8° F.).

Tests in Wyoming (14, pp. 13-16) showed that in moist air the combs froze at 6° F.; at 8° to 10° egg production was reduced 25 percent; and at 0° the hens stopped laying. Other tests reveal that in an uninsulated house a temperature of 7° to 10° higher than that outside may be obtained, whereas in an insulated and ventilated house the difference may be as great as 16° to 30°.

Ruckman (31) found, in a large poultry feeding plant, that at a temperature below 60° F. the consumption of feed per pound of gain increased; and that at 65°, although the birds were healthy, they showed a tendency to lose their appetites and failed to gain. He also found that too high humidity made breathing difficult and too low humidity produced a dry skin and a poor growth of feathers.

The ventilation problem requires a consideration of carbon dioxide and moisture production and removal. Many writers state that it is desirable to maintain a purity of 9 parts of CO₂ to 10,000 parts of air. Purity of air is always to be desired, if it can be obtained without harmful drafts. A higher percentage of CO₂ than that mentioned above may be maintained in the henhouse without apparent harm, but too high a concentration of CO₂ in the incubator room may mean a decreased hatch. At the Iowa Agricultural Experiment

⁵ This is still above the estimated critical temperature of a 4-pound White Plymouth Rock bird, which is 20° F. (table 4).

Station (9) it was found that 11 parts to 10,000 could be obtained with a circulation of 4.2 cubic feet of air per hour per bird, which in this case was approximately 0.3 dilution of air per hour.⁶

There is some doubt as to the amount of water that can be removed from the litter by ventilation. Much depends upon the floor temperature, the vapor pressure balance of the air entrapped in the litter and that just above, and the depth and kind of litter.

The quantity of air required for the removal of the average production of moisture will vary with variations in the air temperature and the relative humidity. Since it has been shown that 4.2 cubic feet of air per hour are required to maintain what may be considered a desirable limit of CO_2 , we can now ascertain the amount of air necessary to remove the moisture at an assumed temperature of 50°F . and 75 percent relative humidity within the henhouse and 15° and 80 percent humidity of the admitted air. Air at 50° with a relative humidity of 75 percent holds 3.057 grains of moisture per cubic foot and the outside entering air would bring in 0.789 grain. Hence to remove 7,315 grains per hour would require $7,315 \div (3.057 - 0.789) = 3,225$ cubic feet per hour, or 16 cubic feet per hour per bird. One British thermal unit will heat 53 cubic feet of air 1° . Hence it will require 2,128 B.t.u. per hour to heat 3,225 cubic feet of incoming air to the temperature of the henhouse.

A 4-pound White Plymouth Rock bird produces 238 calories of heat daily. Two hundred such birds would therefore produce 7,866 B.t.u., hourly. However, only 6,765 B.t.u. are dissipated as sensible heat, the remaining 14 percent being lost in the vaporization of water. The air necessary to remove the moisture produced requires 2,128 B.t.u., leaving a remainder of 4,637 B.t.u. to take care of the heat losses from the house.

The above-mentioned house has an equivalent surface area in walls and roof of 1,336 square feet and 50 square feet of glass. If the walls are insulated to provide a loss of only 0.22 B.t.u. per square foot per degree of difference in temperature per hour, there would be a total loss of 294 B.t.u. from the wall plus 55 B.t.u. from the single glass window, or a total of 349 B.t.u. per hour per degree of difference in temperature.

Thus the heat available would permit the maintenance of 13.3° difference in temperature, whereas under the foregoing assumption a difference of 35° was desired. If the insulation value were increased and storm windows used, a greater temperature difference would be obtained. Moreover, if larger quantities of feed were consumed more heat would be produced, but it might be more economical to provide artificial heat in order to maintain the desired temperature. It should also be noted that no allowance of feed energy for egg production has been made.⁷

The foregoing example shows that it is impractical to maintain a temperature of 50° in such weather and provide the amount of ventilation mentioned without the use of artificial heat. If the results of the Iowa tests are accepted, less ventilation can be used without producing harmful concentrations of CO_2 . However, under

⁶ On the other hand, in respiration experiments animals may be maintained in apparently normal condition in chambers containing as high as 0.5 to 1 part of CO_2 per 100. In the laboratory of the senior author experiments with poultry have been carried out for many days in succession with such concentrations of CO_2 prevailing.

⁷ If the 200 hens were producing 100 eggs daily, their heat production will be increased 2,300 cal. daily or about 342 B.t.u. hourly. This extra heat would permit another degree difference in temperature between henhouse and exterior.

the temperature conditions assumed above, less ventilation would hardly be adequate for moisture removal. The removal of moisture is a major problem in poultry houses. Poultry have no sweat glands, but they give off relatively large amounts of vapor in respiration and through the skin.

It was found at the Nebraska station (32) that maximum egg production was obtained when temperatures were not permitted to fluctuate widely. A henhouse temperature of 50° F. is too high to be maintained on most farms in winter without artificial heat. Hence a lower temperature held uniformly would be desirable. Increasing numbers of poultrymen have had success with artificial heat properly regulated, but failure has commonly resulted when temperatures were allowed to go too high or to fluctuate widely.

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STEWART'S WILT DISEASE OF CORN, WITH EMPHASIS ON THE LIFE HISTORY OF PHYTOMONAS STEWARTI IN RELATION TO PATHOGENESIS¹

By S. S. IVANOFF ²

Research associate in Plant Pathology, Wisconsin Agricultural Experiment Station

INTRODUCTION

The studies reported in this paper were made on certain phases of Stewart's wilt disease of corn, caused by *Phytomonas stewarti* (Smith) Com.S.A.B., particular emphasis being placed on the life history of the causal organism in relation to pathogenesis. The difficulties encountered in studying certain stages of the life history of this organism and in making isolations from badly contaminated material and from the soil, made the development of a selective medium highly desirable. Such a medium was developed through studies of the physiology of the causal bacteria. Other bacterial forms causing symptoms similar to those produced by the Stewart's wilt organism are considered briefly. Likewise, some symptoms produced by the wilt organism, not hitherto described or only partly described, are reported.

Four different phases in the life history of the pathogene in relation to pathogenesis are considered: (1) Its entrance into the host plant, (2) its location in the tissue of the host, (3) its exit from the host, and (4) its mode of transmission to a new host.

EARLIER WORK

The first published report of Stewart's wilt disease of corn appeared in 1897 (16).³ The only known host of the wilt-disease organism is the corn plant, *Zea mays* L. It attacks several kinds of corn but its preferred host is sweet corn. Studies on varietal susceptibility have been carried out by several workers, including Reddy (10), Rand and Cash (8), Reddy and Holbert (11), and Holbert, Elliott, and Koehler (4).

The wilt disease is rather widely distributed in the United States. It is found throughout the central and eastern sections and recently has been reported from the Pacific coast. So far as the writer is aware, this disease is not known to occur outside of continental United States and Puerto Rico. Its economic importance as evidenced by the losses which it causes is great at times. Occasionally an entire corn crop has been ruined, and losses of from 20 to 40 percent have been frequent (16). Unpublished reports estimate the losses in the summer of 1931 and 1932 at certain localities as high as 90 percent of the entire crop of the Golden Bantam variety.

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³ Reference is made by number (italic) to Literature Cited, p. 769

Certain symptoms of the disease have been described by Stewart (16) and Smith (15). They found that the leaves of diseased plants wither gradually. The stem shows no external symptoms, but when cut lengthwise the fibrovascular bundles appear as yellow streaks in the white parenchyma. If the stem is cut crosswise, a yellow viscid bacterial exudate soon oozes from the vascular bundles. This is considered by Stewart and Smith the most characteristic symptom of the disease. The male inflorescence develops prematurely and becomes dry. The whole plant is stunted and bears imperfect, small ears.

The causal organism of this disease was described by Smith as *Pseudomonas stewarti* (12). Later it was renamed *Aplanobacter stewarti* (E. F. Smith) McCulloch (6). According to Bergey the name is *Phytomonas stewarti*.

The physiology of *Phytomonas stewarti* has been studied by Smith (13, 15). The work on its life history in relation to pathogenesis has been done mainly by Smith (14, 15), Rand (7), and Rand and Cash (8, 9). The bacteria enter the host plant, according to Smith (15), chiefly through the water pores of young seedlings. The exit of the bacteria from the host plant has been reported as taking place in the form of exudate from the stomata of the husks (15, 16). The transmission of the bacteria to a new host was found by Smith (14) to take place through infected seed and by Rand (7), and Rand and Cash (8, 9), through the agency of aboveground insects.

The soil relations of the causal organism have received but slight attention. So far no mention has been found in the literature of the isolation of the bacteria from the soil.

MATERIALS AND METHODS

The culture of *Phytomonas stewarti* used in this investigation was isolated from a diseased sweet-corn plant obtained from Dr. A. G. Johnson, Washington, D.C. This culture was purified by several platings and single-cell isolation and its identity established by studies of its physiology and pathogenicity.

Several varieties of maize were used in this work. The sweet-corn varieties included Golden Bantam, which is particularly susceptible to Stewart's wilt disease, Golden Sweet, and Country Gentleman. Two hybrids of sweet corn, known as "hybrids 66 and 70", were also used. Hybrid 66 had been found by G. M. Smith, from whom it was obtained, to be more or less resistant to the disease, and hybrid 70 very susceptible. The field-corn varieties used in some of the trials were Golden Glow, Leaming, and an unnamed variety of dent corn. Unless otherwise stated, Golden Bantam sweet corn was used throughout the trials.

Most of the sweet-corn seed employed in the tests was grown on the experimental plots of the Wisconsin Agricultural Experiment Station. The field-corn variety, Golden Glow, was obtained from Dr. J. G. Dickson and N. P. Neal, of the Wisconsin station, the Leaming variety of field corn was grown by Dr. J. R. Holbert at Bloomington, Ill.; the unnamed variety of dent corn was purchased from a local seed firm.

The amount of diseased and surface-contaminated kernels in a sample of seed was determined by the poured-plate method. Most

of the inoculations were made by means of needle punctures at the base of the plant.

Isolations of the pathogene from the host tissue were made in the usual manner. The medium commonly employed for the isolation of the organism from diseased host tissue, when it was believed to be present in abundance and relatively free from contaminants, was nutrient dextrose agar. For making isolations from badly contaminated diseased material, a selective medium, described later in the paper, was employed. The identity of the organisms secured was determined both by cultural and pathogenicity tests.

Material for histological studies was collected from plants grown in the greenhouse and field and inoculated by the method already mentioned. Since it was found through isolation studies described later that not all of the kernels harvested from diseased corn plants harbored the bacteria within the kernel tissue, a method was employed by which the presence of the bacteria within the tissue of the kernel was established before the material was fixed. This method was as follows: The kernels which were supposed to be diseased were surface-sterilized and placed in sterile Petri dishes, one kernel in each dish. By means of a scalpel a bit of tissue from the tip of the kernel was cut off. The remaining portion of the kernel was removed to another container, labeled, and saved. The excised portion was then soaked in water in the dish and later crushed. Dilution plates were poured in the usual way. Only kernels from which the bacteria were recovered in this manner were used; the others were discarded. This method was based on the fact, established by Smith (15) and later confirmed in these studies, that the bacteria in the tissue of a diseased kernel are at the base of the kernel. Most of the kernels used for histological studies were in the milk stage, since it was found that such kernels were much easier to section.

The fixative most commonly employed for all diseased material was formal acetic alcohol. It destroyed the chondriosomes present in the plant tissue and obviated any possible confusion of these bodies with the bacteria. The fixed material was dehydrated in alcohol, treated with chloroform, and infiltrated and embedded in paraffin in the usual manner. Sections were made 10 to 30 microns thick. Flenning's triple stain was ordinarily employed.

SYMPTOMS OF THE DISEASE

Some symptoms not hitherto described, or only partly described, were observed in plants inoculated by means of needle punctures and grown in the greenhouse and in the field.

The appearance of water-soaked and discolored stripes along the veins of leaves of naturally infected or artificially inoculated plants has been noted by other investigators (2, 5a, 7, 9, 15) (fig. 1, A). The manner of their formation and development, however, as well as the microscopic symptoms of the affected tissue seem to deserve greater attention than they have yet received.

The development of these stripes a few days after puncture inoculations at the base of young leaves was found to be as follows: The first visible symptom, which usually appeared about 3 days after inoculation, was the yellowing of the vein and a slight discoloration of the parenchymatous tissue along the affected vein for some distance



FIGURE 1.—Leaf symptoms of Stewart's wilt disease of corn: *A*, A diseased field-corn plant artificially inoculated in the stem with Stewart's wilt bacteria, showing several necrotic stripes along the veins, *B* to *F*, five stages in the development of these stripes; *B*, discolored veins which appear 2 or 3 days after inoculation; *C* and *D*, water-soaked, semicircular or irregularly shaped areas which appear 2 or 3 days after inoculation; *E*, a stripe along an affected vein formed by the coalescence of the individual water-soaked areas; *F*, several water-soaked stripes which may unite to form a wider stripe upon the blade.

above and below the needle prick (fig. 1, *B*). This was usually followed a day or two later by the appearance here and there on either side of the affected vein of small, water-soaked, semicircular or irregularly shaped areas which appeared to have their centers at the vein (fig. 1, *C* and *D*). Subsequent histological studies showed that these water-soaked areas were near the points at which the bacteria escaped from the vessels into the parenchymatous tissue. Several of these areas coalesced shortly after they appeared and formed an irregular water-soaked stripe along the vein (fig. 1, *E*). Two or more stripes of the same blade in time might unite and produce a long discolored area along several of the veins until the whole blade became involved (fig. 1, *F*). The tissue of the semicircular water-soaked areas as well as that of the water-soaked stripes often lost its chlorophyll entirely, and it then appeared as a semitransparent membrane, which turned light brown with age. These areas were bordered by discolored tissue due to the partial disappearance of the chlorophyll, the discoloration being greatest near the border of the water-soaked region and gradually diminishing away from it (fig. 2, *E* and *F*). The discolored tissue immediately surrounding the water-soaked areas at first appeared slightly lighter than the normal green tissue. Later it turned dull gray, yellow, and light brown in the order named. The discoloration of the diseased tissue did not seem to advance in a regular line, as small dull-gray areas appeared intermingled with light-green or dark-green areas, giving the diseased tissue a mosaic appearance.

These stripes were not always continuous along an affected vein. Occasionally they were interrupted or gradually thinned out and appeared again farther along the same vein. The vascular tissue connecting the two water-soaked areas might or might not be discolored (fig. 2, *A*).

The same characteristic stripes appeared when the plants were inoculated at the root, stem, or midrib, but when the inoculation was made on the roots or stem the symptoms appeared after a relatively longer time. When the inoculation was made at the base of the midrib of an unfolding blade no symptoms were visible in the midrib, but from the points at which the numerous colorless vascular bundles traversing the midrib begin to separate from it and spread into the green tissue of the leaf, the characteristic water-soaked stripes appeared along the veins and gradually extended toward the tip of the leaf. In such cases the leaf appeared to wilt from the tip downward (fig. 2, *B* to *D*). Similar cases were observed when inoculations were made through the stem of the plant and the affected bundles extended for some distance through the midrib of the leaves.

The leaf symptoms seemed to vary with the host plant. On the leaves of the dark-green varieties of field corn the stripes were usually wider and more pronounced than on those of the lighter green varieties of sweet corn (fig. 2, *E*). Correspondingly, the leaves of the dark-green commercial variety of dent corn turned light brown and died sooner than those of Golden Bantam when both were grown and inoculated at the same time and under similar conditions.

Since the stripes appeared after wound-inoculation of young plants, they aided considerably in the early location of diseased plants, both in the greenhouse and in the field. When mature plants were inoculated these leaf symptoms were not observed. Similar leaf symptoms were produced when young corn plants were inoculated in the

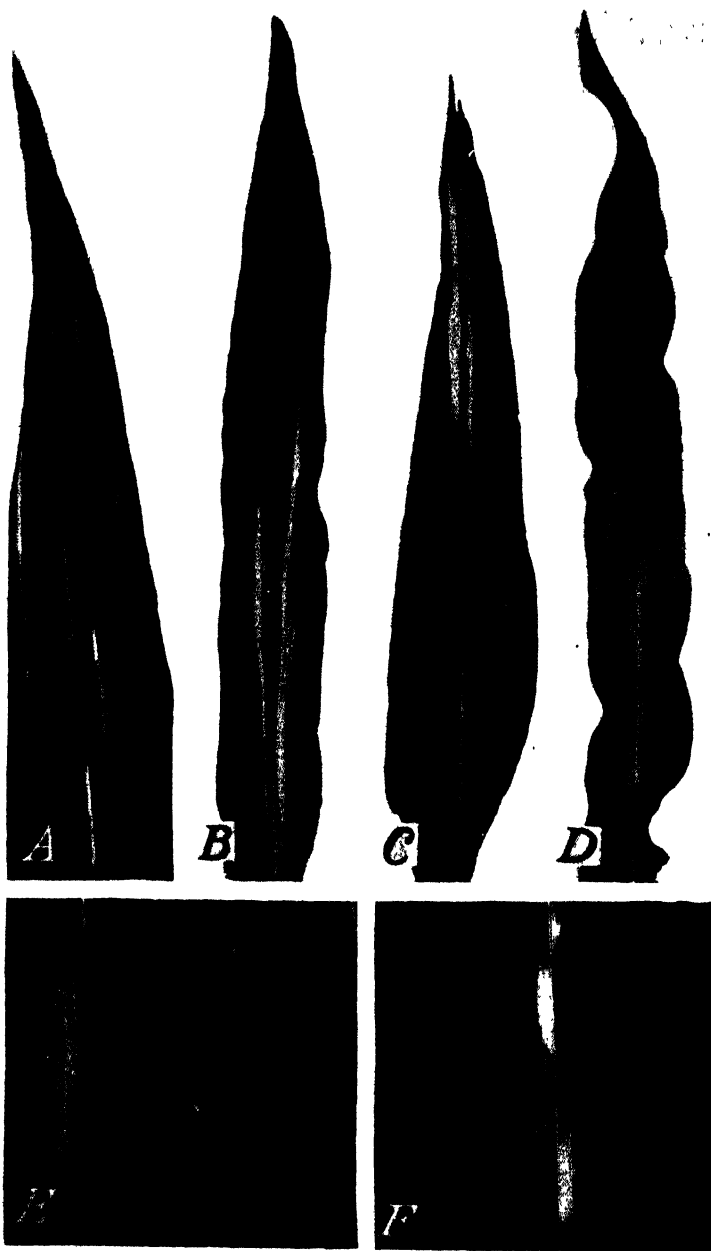


FIGURE 2.—Leaf symptoms of Stewart's wilt disease of corn: *A*, Stripes on a blade of Golden Bantam sweet corn in their various stages of formation. Leaf stripes are often discontinuous, as shown in this specimen. *B* and *C*, Leaves showing the characteristic stripes along veins which have separated from the midrib. The plants from which these leaves were taken were inoculated in the stalk. *D*, Same as in *B* and *C* but of a later stage, showing dried portion of the leaf extending from the tip downward. *E*, A wide, well-pronounced stripe on the blade of a dark-green variety of field corn. The numerous small dark spots on the stripe represent water-soaked areas in the parenchymatous tissue. $\times 3$. *F*, Water-soaked areas adjoining an affected vein of a blade of a sweet-corn plant. Discolored tissue surrounds the water-soaked areas. $\times 4$.

same manner with other bacterial cultures which differed in certain cultural and physiological characters (3, 5) from the standard culture of *Phytophthora stewarti* used throughout this work.

Bacterial exudate was often observed upon the water-soaked or necrotic stripes of young seedling leaves. Most commonly it appeared in the form of small bright-yellow drops. In a few instances cream-colored drops of bacterial exudate were observed apparently exuding from the interior of the kernels. These kernels were found on anomalous tassels, part of which had assumed the form of pistillate spikes which produced fruit. As is usual in such cases, the kernels were not covered by husks, and there was no danger of their having been contaminated by the bacterial exudate commonly found on the inside of husks. After the exudate had been removed a slit in the kernel tissue, just beneath the bacterial drop, was visible.

Badly diseased cobs failed to develop normal kernels. Occasionally one side of a cob was found to be more seriously affected than the other. In such cases the kernels on one side of the cob developed to normal size while those on the other side were small, shriveled, or did not develop at all.

LIFE HISTORY OF THE CAUSAL ORGANISM IN RELATION TO PATHOGENESIS

The life history of *Phytophthora stewarti* in relation to pathogenesis was given especial attention. As stated above, four different phases in the life history of the organisms were considered—entrance, location, exit, and transmission.

ENTRANCE OF THE ORGANISM INTO THE HOST TISSUE

Several possible ways by which the bacteria might enter the host plant were studied. These were entrance (1) through the broken pericarp at the time of germination, (2) through unwounded roots, (3) through punctured roots, (4) through bruised and wounded roots from infested soil, (5) through wounds made by insects feeding upon the roots from infested soil, and (6) through stomata and water pores of young corn plants.

The ruptures which are made in the pericarp by the emerging primary root at the time of germination were tested as infection courts. Three tests were conducted, 2 in the laboratory and 1 in the greenhouse. In 1 laboratory test about 50 healthy kernels were surface-sterilized and placed upon the surface of hardened nutrient agar in Petri dishes, the embryo side up, 1 kernel to a dish. After the kernels had imbibed water and swelled overnight, and before the coleorhiza had emerged through the pericarp, a small drop of bacterial suspension was applied to the tip of each kernel. When the coleorhiza emerged through the pericarp, and the primary root tip appeared through the broken root sheath, there was already bacterial growth on the agar around and in contact with the basal edge of the kernel. As the primary root continued to grow and form branch roots and root hairs, the bacterial growth increased, finally completely surrounding all the roots and root hairs and partly enveloping the remnants of the kernel. The plumules appeared uncontaminated by the bacteria. Two weeks after germination no symptoms of disease appeared on the leaves of any of the seedlings. Subsequent histologic examinations of fixed specimens revealed masses

of bacteria between the pericarp and the tissue of the broken coleorhiza, between the coleorhiza and the epidermis of the primary root, and in the ruptured cells of the root sheath, but no bacteria inside the tissue of the young plant. The roots of some of the seedlings were then punctured with a needle. Four days later the characteristic water-soaked stripes appeared in the leaves of the punctured plants. In another trial, similar to the one just described, 42 healthy kernels were soaked in a bacterial suspension overnight and then placed on the surface of nutrient agar slants in large test tubes, where they grew in the absence of other organisms for 3 weeks. Although the bacteria grew profusely on the agar medium, none of the plants became infected. Subsequent isolations from the leaf tissue of each plant did not yield any bacteria. Similar greenhouse trials having the same object produced similar results.

Entrance of the pathogene into wound-free roots has not been found to occur under the conditions employed. To determine whether the bacteria gain entrance to the host plant through wounded roots and other injured underground parts of very young corn seedlings, two sets of experiments were conducted in the greenhouse. Uninfected seed was germinated under conditions which excluded any contact between the seedlings and the wilt bacteria. Four days after germination the seedlings were divided into two groups and given special treatment before they were planted in autoclaved soil. This treatment was as follows: Each seedling of the first group was painted by means of a camel's-hair brush at the primary root, at the broken coleorhiza, and at the lower part of the kernel, including the scutellum, with a suspension of the wilt organism. Some of these seedlings were punctured at the primary root by means of an inoculating needle and others were pricked at the scutellum by passing the needle through the region of the cotyledonary plate; the rest received no treatment. The seedlings of the second group were treated in a similar manner, except that sterile water instead of bacterial suspension was used. After these treatments all the seedlings were planted in holes dug for them in the bed. A week after planting, the characteristic water-soaked stripes appeared on the leaves of some of the seedlings that had been painted with bacterial suspension and punctured with a needle. Seedlings that had not been so treated showed no symptoms of the disease. The water-soaked streaks of the affected plants appeared to progress upward from the injury along the veins of the leaves.

Several experiments were conducted to determine whether infection could take place through wounds on the roots of plants growing in infested soil. The plants for these experiments were grown in boxes so constructed that special treatments and observations on the roots could be made. The boxes were about 20 inches long, 9 inches wide, and 13 inches deep. Two sides were removable. Under each of these removable sides there was fitted a glass plate to permit observation of the roots. Eighteen of the boxes were filled with soil, steamed, and seeded with bacteria-free corn, about 12 kernels to the box. When the plants were 2 to 3 weeks old the boxes were divided into 4 series for different treatments, as follows: The soil of the first series, consisting of 6 boxes, was infested with a suspension of Stewart's wilt bacteria, and the roots of the plants were wounded by running a dull scalpel through the soil. The soil of the second series of five boxes

was also infested with the bacteria, but the roots were left undisturbed. In the third series, consisting of four boxes, the roots were wounded and the soil was watered, but no bacteria were introduced. In the last series, consisting of one box only, the roots were left undisturbed and the soil free from wilt bacteria. Seven to fourteen days after the treatment the characteristic water-soaked stripes appeared on the leaves of some of the plants. The final results were taken about a month after the treatment. In the first series some plants in all of the 6 boxes were diseased, the percentage of diseased plants per box ranging from 18 to 100. In the second series, all of the plants in 3 of the boxes remained healthy but in 2 boxes there were 1 or 2 diseased plants, owing probably to accidental wounding of the plant roots in handling the boxes. In the boxes of the third and fourth series, where wilt bacteria were not introduced, no diseased plants were observed. These experiments were repeated 3 times, more than 500 plants in all being used.

In another experiment, white grubs, the larval stage of *Phyllophaga* sp., were used as agents for the wounding of the roots in soil artificially infested with the wilt bacteria. These insects were selected for wounding the roots because they commonly feed on roots of corn plants. In carrying out these trials the same materials and methods were used as in the experiments just described, except that instead of wounding the roots with a scalpel the white grubs, collected from under the sod, were placed on the surface of the soil of several boxes. A summary of the results of repeated trials with these insects showed that 50 percent of the plants became infected.

It was thought that the bacteria might enter through stomata and water pores of young corn plants. Accordingly, about 100 healthy kernels were surface-sterilized and planted on a bench in the greenhouse in autoclaved, insect-free soil. After the plants had reached the second- and third-leaf stage 60 were inoculated by placing a drop of bacterial suspension on the tip of each leaf. Thirty of the remaining plants were treated in a similar manner except that instead of bacterial suspension, sterile water was used. Four other plants were inoculated by placing a drop of the bacterial suspension at the base of the plant and a needle puncture made through it. Another 4 plants were similarly treated, except that sterile water was used instead of the bacterial suspension. The last three treatments served as controls. The temperature of the house at the time of these treatments and for a few days following was approximately 70° F. The plants were kept under observation for more than a month but no symptoms of disease were noted except on plants inoculated with the bacterial suspension by means of needle punctures. Before the plants were discarded a test was made for the presence of the bacteria within the plant tissue by plating out leaf tissue from a few of the plants which had previously had drops of bacterial suspension at their leaf tips. No wilt bacteria were recovered. A similar experiment was performed later in the season, and approximately similar results were obtained. In this experiment about 100 plants were grown in pots with sterilized soil. The inoculation was made when the plants were in the second- and third-leaf stage by placing 1 cc of bacterial suspension in the funnel formed by the uncurling leaves. The plants were kept in the humid atmosphere of the greenhouse for 5 days at a temperature of about 70° F. They were then taken out of the greenhouse.

No symptoms had appeared on any of the plants by the end of the third week after inoculation. Final observations were made at the end of 6 weeks, and it was found that 1 plant had a water-soaked stripe typical of Stewart's wilt disease. Since the stripe arose from below the soil level and appeared long after the usual incubation period for this disease, it was thought probable that infection had taken place through the roots at a time considerably after the plants were inoculated.

LOCATION OF THE BACTERIA IN THE HOST TISSUE

The location of the pathogene in the plant tissue has been studied by Smith (15), as previously mentioned. Many of his findings were verified in the present work and in some instances extended.

In the leaves the bacteria were located in the vessels of the vascular bundles, in large cavities adjoining disrupted bacteria-filled vessels, in the intercellular spaces, and substomatal cavities of the parenchymatous tissue, and as exudate, upon the surface of the blade.

In the vessels the bacteria were found in great masses, in many cases completely filling these structures. Gum material was also observed in the vessels. Its origin and the manner of its formation require further study. Often when a piece of diseased tissue was placed near the edge of a water drop, the drop would quickly become clouded with the bacteria, which would continue for several minutes to flow out from the vessels into the water drop.

In the parenchymatous tissue of the leaf, bacterial pockets or cavities were found adjacent to the openings in the walls of the vessels. These cavities were very numerous and appeared along one side, or more often, along both sides of a bacteria-filled vessel. They were of various sizes, reaching at times a diameter of one half millimeter or more and were invariably filled with large gummy masses of bacteria. These cavities seemed to correspond to the water-soaked semicircular areas, already described as occurring on either side of a vein a few days after inoculation (fig. 1, *C* and *D*, and fig. 2, *E* and *F*). The parenchymatous cells near the openings at the vessel wall were apparently destroyed by the outflowing mass of bacteria. There were some ruptured parenchymatous cells visible all around the bacterial cavities which were also filled with bacteria. Adjacent to these ruptured cells were several rows of other parenchymatous cells which appeared plasmolized and collapsed. These areas of diseased tissue were found to correspond to the discolored areas of the leaves described on page 753. The middle lamellae of some of these cells seemed to have been dissolved by the bacteria, which were found between the cells and in the intercellular spaces of the tissue (pl. 1, 41). The discoloration of the cells gradually diminished as the number of bacteria between the cells decreased. These areas apparently corresponded to the dull gray or lighter green portions of the characteristic stripes on the leaves described above.

The exact manner in which the bacteria make their way out of the vessels into the parenchymatous tissue has not been determined.

In many of the substomatal cavities of the slightly discolored tissue adjacent to the necrotic leaf areas, masses of bacteria were observed with the microscope. Bacteria were also found on the characteristically wrinkled epidermal cells above these cavities. These bacteria, however, were not present in sufficient quantity to be seen

as exudate on the surface of the leaf. The bacterial exudate was found to come not only from the substomatal cavities but also directly from large, bacteria-filled vessels from which the bacteria made their way out as yellow drops visible to the naked eye through longitudinal slits in the walls of the vessels and the overlying leaf tissue.

In the husks the location of the bacteria was similar to that in the leaves. In some cases small drops of gumlike deposits, which took the blue of Flemming's triple stain, were found between the cells intermingled with the bacteria and in the intercellular spaces (pl. 1, *B*). Whether these deposits were produced by the bacteria or represented host reaction to injury, as is the case with some other diseases, was not determined.

In the stem, shank, and cob the bacteria were found in the vessels, in the large air spaces of some of the bundles, in masses in the disrupted pith tissue, and as an exudate on the surface of the shank. Many of the vessels were filled with bacteria. By making openings in the walls of the vessels the bacteria find their way into the large air space which most of the centrally located bundles of the stem have (pl. 1, *C*), and also into the adjoining sclerenchymatous and parenchymatous cells, where they progress between the cells. In the air spaces of the bundles the bacteria were found in great masses, considerably enlarging these spaces apparently by destroying the cells of the surrounding tissue.

In the tassel the bacteria were located in the vascular system of the rachises, of the rachillas, and of the glumes, in the delicate spiral vessels of the filaments, and in the parenchymatous tissue of the glumes. The location of the bacteria in the rachises and the rachillas is similar to that in the stem and cob. In the glumes the bacteria were found in practically the same position as in the husks. Here also the bacteria occupied the substomatal cavities and the intercellular spaces adjacent to bacterial cavities caused by openings in the walls of the vessels. In some filaments the spiral vessels were almost completely occupied by bacteria, but no openings in the walls of the vessels were observed. Although these vessels are separated from the pollen chambers by only a few rows of cells, in the specimens examined no bacteria were observed in the pollen chambers. However, bacteria are sometimes found mixed with pollen.

Bacterial exudate similar to that found on diseased young seedling leaves was occasionally observed on the glumes. It exuded from the stomata located both above and below the level of the anthers.

Bacteria were isolated from the glumes, the anthers, and the pollen of diseased plants. These isolations were made from each part at 4 different times from material obtained from at least 3 different plants by removing the various parts of staminate spikelets of diseased plants under aseptic conditions. The isolations from the pollen were made by removing an anther from an open flower by means of sterile forceps and shaking it over a sterile Petri dish, after which a nutrient medium was poured in. It was not clear whether the bacteria were located in the pollen chambers or on the surface of the anthers. It is possible that the pollen had become contaminated by coming in contact with bacterial exudate oozing from the stomata of the glumes.

In the kernel the bacteria were located in the vascular system and parenchymatous tissue of the chalazal region, between the innermost

row of cells of the chalazal region and the marginal layer of the endosperm, and in the endosperm. The bacteria were found in the bands of spiral vessels which form a prolongation of the vascular system of the cob and extend into the tissue of the chalazal region and the pericarp. Some of these vessels were plugged with bacteria. Sometimes openings in the walls of the vessels occurred, resulting in the formation of cavities of various sizes (pl. 2, *A*). Often these cavities attained relatively large dimensions and were surrounded by collapsed cells. Frequently remnants of broken vessels were found mixed with the mass of bacteria (pl. 2, *B*). The number of these cavities varied in different kernels. In some kernels as many as 18 were observed in one section. All of these cavities were found in the parenchymatous tissue of the chalazal region, a few rows of cells from the endosperm. None were seen in the adjacent spongy tissue. In a few instances some of the parenchymatous cells of the chalazal region were badly ruptured, masses of bacteria lying among the broken cells (pl. 3, *A* and *C*). Through these ruptures the bacteria apparently found their way between the tissue of the chalaza and the marginal layer of the endosperm, where they were observed in masses embedded in slime. The further advance of the bacteria was followed through the ruptured aleurone layer to the starchy endospermic tissue where the bacteria lay between the cells in masses of slime (pl. 3, *B*).

EXIT OF THE BACTERIA FROM THE HOST PLANT

The exit of the bacteria from the host plant was studied by macroscopic observation and by histological and cultural technic.

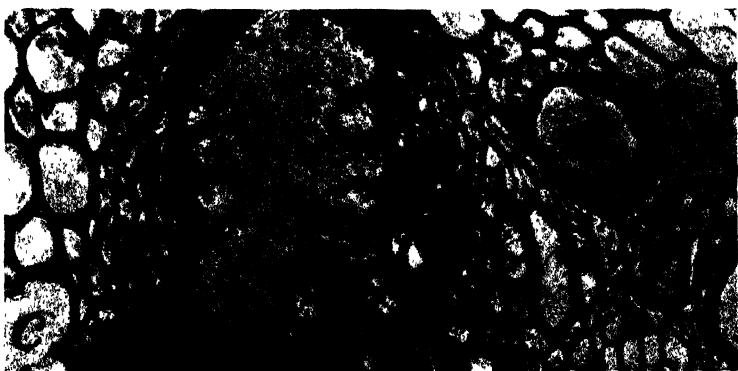
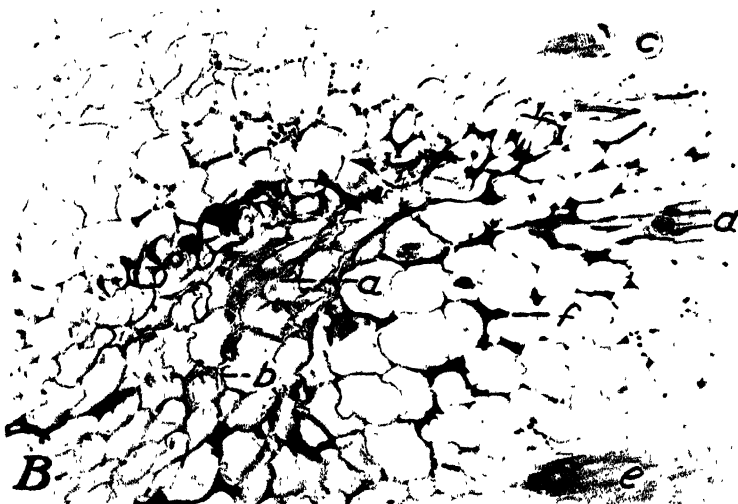
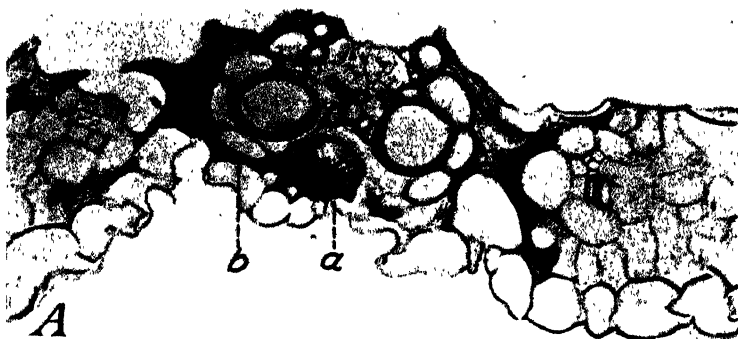
The exit of the bacteria from the host plant as exudate from diseased leaves, shanks, husks, glumes, and kernels has already been mentioned. This exudate is apparently washed from the diseased plant parts and carried with the rain water to other plant parts and also into the soil. The recovery of the bacteria from other plant parts, as well as from artificially infested soil and from dead plant material, was accomplished by employing a selective medium, described later in this paper.

The wilt organism was isolated from the water which accumulated between the blade and the ligule and at the base of young uncurling leaves of plants grown in the greenhouse and outdoors. Such isolations were made at two different times, three plants being used at a time. The aggregation of bacteria in these water drops was sometimes considerable, and was undoubtedly due to the washing down of bacterial exudate from diseased leaves by rolling dew drops and falling rain water. The role of this natural bacterial suspension in the initiation of the secondary cycles of the disease was not studied.

The bacteria were isolated without much difficulty from disintegrating plant parts taken from above and below the ground.

(1) From corn stubble of diseased plants which had overwintered in the field the bacteria were obtained in the following spring at planting time. The recovery of the bacteria from the stem tissues was made by crushing the tissues in a test tube of sterile water after which dilution plates were poured from the suspensions with the selective medium.

(2) From decaying roots and stems in 4 out of 6 cases the bacteria were recovered a few months after the crop had been harvested, by



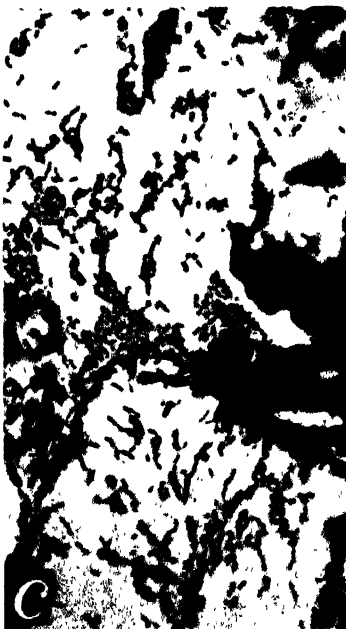
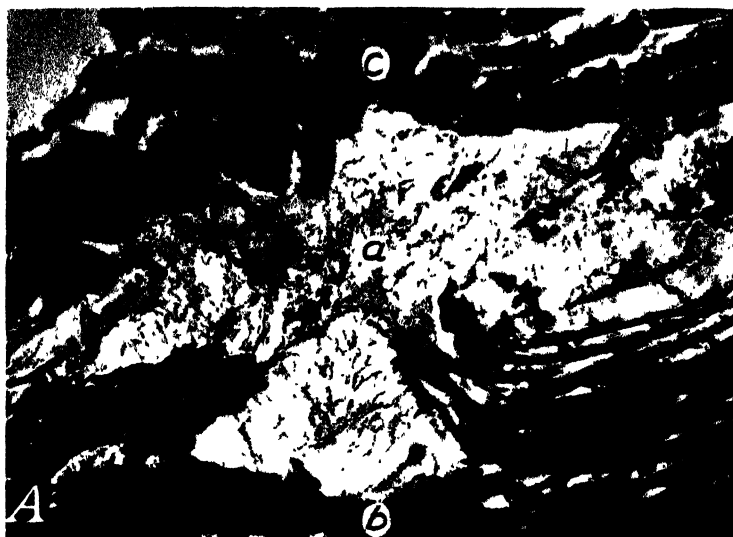
LOCATION OF STEWART'S WILT BACTERIA IN THE TISSUES OF CORN LEAF, HUSK, AND STEM.

A, Cross section through a water-soaked strip of a diseased leaf showing the general collapse of the tissues. The bacteria may be seen occupying one of the vessels as well as the intercellular spaces at *a* and *b*. $\times 340$.
B, Horizontal section through husk tissues beneath a drop of bacterial exudate. The bacteria may be seen in cavities in the parenchymatous tissue at *a* and between the cells at *b*. At the right of the illustration are three vascular bundles, *c*, *d*, and *e*, the middle one extending through the bacterial cavity. The wall of one of the vessels of this bundle had an opening near the cavity. Gumlike deposits may be seen in the intercellular spaces surrounding the bacterial cavity as at *f*. $\times 100$.
C, Cross section through the tissues of a diseased stem. The bacteria are shown in several vessels of a vascular bundle and in the cavity of the large air space of the bundle. $\times 310$.



LOCATION OF STEWART'S WILT BACTERIA IN THE CORN KERNEL

1. Bacterial cavities of various sizes, the largest shown at *a*, situated in the chalazal region of a kernel. The endosperm is shown to the right of these cavities, at *b*. Some of the vascular tissue of the kernel is shown at *c*, adjoining a bacterial cavity. $\times 80$. *B*, Enlarged view of a bacterial cavity situated in the chalazal region of a corn kernel. Remnants of spiral vessels in the cavity are shown at *a*. The cells near the border line of the cavity at *b* appear to have collapsed. $\times 290$.



LOCATION OF STEWART'S WILT BACTERIA IN THE CORN KERNEL

A, Ruptured tissue in the chalazal region with bacteria in the cavity at *a*. The tissue of the chalaza appears at *b* and that of the endosperm at *c* $\times 350$. *B*, Bacteria, at *a*, embedded in slime between cells of the endosperm, which is shown at *b*. The tissue of the chalaza is shown at *c* $\times 350$. *C*, An enlarged portion from *B* showing individual bacterial cells together with remnants of ruptured host tissue. $\times 645$

using the selective medium. An attempt to isolate the bacteria from these decaying plant parts, by employing common laboratory media had previously been made without success.

(3) From disintegrating parts of six diseased kernels the bacteria were isolated a few days after the kernels had germinated in the soil. The survival of the bacteria in the kernel after germination was thought to be important in relation to the transmission of the disease through seed. In one of the laboratory experiments it was found that when diseased kernels were germinated on agar plates the bacteria made their exit through the ruptured tissue of the broken pericarp and completely surrounded the roots of the seedling, just as was the case with artificially contaminated seed, described above. Bacteria around the roots, if they do occur in the soil, might afford inoculum for the infection of the young plants after wounding. This phase of the problem requires further study.

(4) From artificially infested soil the bacteria were recovered several days after the infestation by employing the selective medium mentioned above.

TRANSMISSION OF THE DISEASE TO A NEW HOST

The transmission of the disease to a new host was tested in the following ways: (1) Through the soil, (2) through diseased seed, and (3) by transferring insects that had fed on diseased plants to healthy plants.

Experiments on soil transmission were not carried beyond the stage already described (pp. 756-757).

Experiments were conducted in the laboratory, greenhouse, and field to determine the role of infected seed in the transmission of the disease. In the laboratory 22 diseased kernels were germinated on nutrient agar plates. As expected, the bacteria from within the tissue found their way out and produced growth on the agar medium, completely surrounding the roots of the young seedlings. Two days after germination the seedlings were transplanted to two boxes filled with autoclaved soil, 11 plants being set in each box. The roots of the seedlings in one of the boxes were broken before planting; those of the remaining plants were left intact. Ten to 12 days after planting, 10 of the plants, the roots of which were broken, showed symptoms of disease, whereas only 2 of the plants in the other box became diseased. In these two cases it is possible that the roots of the plants were unintentionally broken at the time of transplanting. The number of plants used in this experiment was small because of a lack of infected seed.

The transmission of disease through infected seed was tested in the greenhouse by planting in autoclaved soil 100 kernels collected from diseased plants. Laboratory tests had shown that this particular sample of seed contained 9 percent of infected kernels. The plants were grown until they were 2 months old, but none showed any symptoms of disease. This experiment was carried out on a larger scale in the field by planting more than 500 kernels of seed collected from diseased plants of the previous year. This seed sample contained 4 percent of infected seed. The plants were examined from time to time until they reached maturity, but none was found to be diseased. The roots of a number of these plants were examined for root-chewing insects but none was found. These results are more or less in accord

with those obtained by Rand (7) in his greenhouse experiments with diseased seed. It appears very likely that wounding of the subterranean parts of the host plant at certain stages of its development by insects or other agencies may be an important factor in the transmission of the disease through seed. This phase of the problem requires further study.

Transmission of the disease by above-ground insects has been studied by Rand (7) and Rand and Cash (9). They reported that direct dissemination of the bacteria from leaves of diseased plants to leaves of healthy plants is accomplished through the agency of flea beetles and the 12-spotted cucumber beetle. Experiments on insect transmission were continued by the present writer, but in these experiments below-ground insects were employed.

During the summer of 1932 several thousand plants of several varieties of sweet corn obtained from various sources were grown at the Wisconsin Agricultural Experiment Station. Some of these plants showed the characteristic stripes of Stewart's wilt disease. Upon closer examination it was found that while the percentage of diseased plants was rather small (less than 1 percent), these plants adjoined each other, growing usually in groups of 2 or 3. Some of the diseased plants were removed, and it was found that on the roots or at the base of the stem of each plant from 1 to 3 insect larvae were feeding. Eleven of these were collected and later identified as the corn rootworm (*Diatraea longicornis* (Say)). The stems of the diseased plants when split open showed the characteristic yellow bundles of Stewart's wilt disease. Subsequent isolations from the stems and leaves of these plants and from the larvae produced typical colonies of *Phytophthora stewartii*. The pathogenicity of these isolations was further established by inoculation tests. The role of these insects in the transmission of the disease was determined in the following manner. Each of the 11 bacteria-infested larvae was placed near the base of a young corn plant growing in the greenhouse. Care was taken not to disturb the roots of the plants. Each plant was then surrounded by a large metal cylinder which was thrust about 2 inches below the surface of the soil in order to confine the activities of the insect to one plant only. Six additional insects taken from apparently healthy plants were used in the same manner and served as controls. Bits of tissue of the healthy plants and washings from the control insects yielded no bacterial-wilt colonies on nutrient dextrose agar plates after the usual incubation period. The treated plants were examined daily for symptoms of disease. About 10 days after the treatment, 9 of the plants showed the characteristic leaf symptoms of bacterial wilt. The control plants showed no evidence of disease. Leaf tissue from 5 of the diseased plants and from 2 of the control plants was plated. Typical *Phytophthora stewartii* colonies were obtained in abundance from the tissue of the diseased plants but none from the control plants. During the attempt to recover the insects it was found that the insects on the 2 disease-free plants and 1 of the control insects had died. The rest of the larvae were alive and active, lodged at the base of each plant. The same experiment was repeated four times with the same lot of insects but on a new lot of healthy plants, some of which were grown in the special boxes mentioned previously. The results of these experiments were similar to those of the first. The lateness of the season prevented the use of a larger number of larvae for another series of trials.

DEVELOPMENT OF A SELECTIVE MEDIUM FOR THE DIRECT ISOLATION OF THE ORGANISM FROM THE SOIL

Attempts to isolate the bacteria directly from infested soil, or from badly contaminated plant parts, by employing common laboratory media, such as nutrient dextrose agar, potato agar, and yeast-infusion agar produced no positive results. The rate of growth of Stewart's wilt organism on these media was found to be considerably slower than that of many common soil organisms. Consequently it was outgrown and inhibited. An attempt was made, therefore, to develop, through physiological studies, a laboratory medium that would be more favorable for the growth of the wilt organism than for that of other bacteria and fungi commonly found in the soil. Accordingly, further studies were made in the growth requirements of the wilt organism. Since the object of these studies was to develop a selective medium, some of the tests by necessity were of a gross character, and were considered as preliminary to a more detailed physiological study of the bacteria to be made later. The studies were conducted along three different lines: (1) The influence of the hydrogen-ion concentration and the oxidation-reduction character of the medium upon the growth of the bacteria, (2) the nutrient requirements of the bacteria, and (3) the effect of the various inhibiting agents on the growth of the bacteria.

Certain bacteria and fungi commonly found in soil were run through some of the tests in parallel with the Stewart's wilt organism for the purpose of comparison. These organisms were *Phytomonas rhizogenes* Riker et al., *Bacillus subtilis* (Ehrenberg) Cohn, *Diplodia zeae* (Schw.) Lev., and an unidentified species of soil *Fusarium*.

HYDROGEN-ION CONCENTRATION AND OXIDATION-REDUCTION CHARACTER OF THE MEDIUM

Growth occurred in media having a wide range of hydrogen-ion concentration. A rough determination of the effect of hydrogen-ion concentration upon the growth of the bacteria was made in the usual manner by varying the reaction of nutrient broth containing 10 g of dextrose.

Abundant growth of the bacteria was observed from pH 6.0 to pH 8.0. On the acid side growth decreased at pH 5.5 but was still marked at pH 4.5. On the alkaline side growth decreased at pH 8.5. These determinations were repeated by using nutrient dextrose agar slants, and similar results were obtained.

Preliminary tests showed that highly oxidized media were favorable for the growth of the bacteria. The influence of the oxidation-reduction character of the medium was studied by employing a method described by Allyn and Baldwin (1). This method consisted in oxidizing and reducing certain restricted areas in Petri plate cultures by placing in them blocks of plain agar, adjusted to the proper pH, in which either oxidizing or reducing compounds had been incorporated. The influence of these blocks on the growth of the bacteria was indicated by the marked contrasts in the number of colonies appearing on the treated and untreated portions of the agar plate cultures.

Two media, A and B, were employed. Medium A had the following composition: Glycerol, 30 cc; sodium asparaginate, 10 g; sodium sulphate, 2.5 g; sodium chloride, 2.5 g; potassium phosphate (dibasic), 2.5 g; calcium chloride, 0.1 g; magnesium sulphate, 0.1 g; agar, 17 g;

and water to make a liter. The medium was adjusted to pH 7.0. Medium B was like medium A except that glycerol was omitted.

Three oxidizing compounds were used in the blocks in various concentrations. These were potassium permanganate, 0.1 percent; ferric ammonium citrate, 1 percent; and hydrogen peroxide, 0.001 and 0.002 percent. The reducing materials used were cysteine, 0.2 percent; and ferrous ammonium sulphate, 0.1 and 0.2 percent.

In these tests there was a marked increase in the number of colonies above and in the vicinity of blocks that contained oxidizing compounds and a diminution in the number of colonies above and around blocks that contained reducing compounds. The greatest stimulation of growth appeared above and in the vicinity of blocks that contained 1 percent of ferric ammonium citrate. The control blocks, containing plain agar only, influenced the growth of the bacteria unfavorably. Ferric ammonium citrate in the concentration employed appeared promising.

NUTRIENT REQUIREMENTS OF THE BACTERIA

The studies on the nutrient requirements of the bacteria included tests of various substances (1) as sources of carbon, and (2) as sources of nitrogen.

SOURCES OF CARBON

A number of compounds were tested as sources of carbon. These included glucose, galactose, sucrose, maltose, lactose, starch, pectin, glycerol, and mannitol. The availability of these compounds for growth of the bacteria was tested by adding a definite quantity of each to a basic medium, which in this case was the sodium asparaginate mineral salts medium (B) already described. All of the compounds except glycerol were added to the amount of 1 percent; glycerol was added to the amount of 3 percent. After sterilization, each medium was adjusted to pH 7.0 and tubed under aseptic conditions. Two or three agar slants of each medium were then seeded with a 48-hour-old culture of the wilt bacteria grown on nutrient dextrose agar slants. For checks the last-named medium was used, as it was found to be very favorable for growth of the bacteria. The cultures were examined at the end of 2-day and 7-day periods. The results showed that most of the compounds used, including mannitol and glycerol, supported growth of the bacteria, which approximated in amount that on the nutrient dextrose agar slants. The relative availability of glycerol and mannitol to the bacteria was further tested by the poured-plate method. One cubic centimeter of a standardized bacterial suspension was placed in each of a number of Petri dishes and the medium poured in the plates. A subsequent count of the bacterial colonies showed the glycerol medium to be considerably more favorable for growth of the bacteria than the mannitol medium. Employing the same technic but using soil solutions instead of suspensions of the wilt organism, the two media were tested in relation to soil bacteria and fungi. The results showed that fewer of the soil micro-organisms grew on the glycerol medium than on the mannitol medium. Consequently glycerol was later selected as a source of carbon in the selective medium.

SOURCES OF NITROGEN

The following salts were tested as sources of nitrogen: potassium nitrite, potassium nitrate, ammonium sulphate, sodium asparaginate, asparagine, and sodium taurocholate. Yeast infusion was also tried as a source of nitrogen. The basic medium in which these substances were incorporated had the following composition: Glycerol, 30 cc; sodium sulphate, 2.5 g; sodium chloride, 2.5 g; potassium phosphate (dibasic), 2.5 g; calcium chloride, 0.1 g; magnesium sulphate, 0.1 g; agar, 17 g; and distilled water sufficient to make a liter. The amount of each nitrogenous material added to the basic medium, with the exception of sodium taurocholate and yeast infusion, was 10 g to the liter. Three grams of the sodium taurocholate was added; yeast infusion was added in the proportion of 100 cc of a 10-percent infusion to 900 cc of the medium. A portion of the basic medium, without a nitrogenous compound, served as a check. The rest of the procedure in these trials was the same as that employed in the carbohydrate tests. The results showed that potassium nitrite did not favor the growth of the bacteria, potassium nitrate and sodium taurocholate produced a slight growth, and sodium asparaginate, asparagine, and yeast infusion produced good growth. The best growth was secured from ammonium sulphate. The other four organisms also grew on the ammonium sulphate medium but none grew so well as the wilt bacteria.

INFLUENCE OF VARIOUS INHIBITING AGENTS ON THE GROWTH OF THE BACTERIA

Several inhibiting agents were tested to determine what influence they might have on the growth of the bacteria. These agents were sodium taurocholate, sodium chloride, and various dyes. The first two compounds were tried singly or in combination in various concentrations in a medium having the following composition: Glycerol, 30 cc; ferric ammonium citrate, 10 g; sodium sulphate, 2.5 g; potassium phosphate (dibasic), 2.5 g; sodium chloride, 2.5 g; calcium chloride, 0.1 g; magnesium sulphate, 0.1 g; agar, 17 g; and water to make a liter. The dyes were tried in various concentrations in nutrient dextrose agar. The various materials were added in the desired concentrations in test tubes with a measured amount of melted agar medium, under aseptic conditions. Agar slants of the medium served as checks.

Sodium taurocholate did not inhibit the growth of the wilt bacteria when added to the medium to the extent of 0.3 percent. In fact, its presence in the medium stimulated the growth of the wilt bacteria, while it completely inhibited that of *Bacillus subtilis*. *Phytomonas rhizogenes* and the two fungi grew well on this medium but not so well as *P. stewarti*.

The effect of sodium chloride on the growth of the bacteria was tested. Smith (15) reported that the wilt organism grew promptly in +15 peptone bouillon containing 5 percent of this salt. In the present studies it was found that sodium chloride did not inhibit the growth of the wilt bacteria when added to the glycerol-ferric ammonium citrate-mineral salts-agar medium to the extent of 2.0 percent. On the other hand, the growth of *Phytomonas rhizogenes* and *Diplodia zeae* was completely inhibited when 1.5 percent of sodium chloride was added to this medium. The bacteria grew in higher

concentrations of sodium chloride when sodium taurocholate was present in the medium.

A number of dyes were found to have no inhibiting effect upon the growth of the wilt bacteria when employed in relatively low concentrations. The dyes tested were basic fuchsin, crystal violet, malachite green, dahlia violet, methylene blue, and thionine. These were employed in concentrations of 1 to 10,000, 1 to 25,000, 1 to 50,000, and 1 to 100,000. Aqueous solutions of these dyes were made and 1 cc of each solution was added under aseptic conditions to 9 cc of nutrient dextrose agar adjusted to pH 7.0. Of the six dyes used, all but one—malachite green—permitted normal growth up to a concentration of 1 part of the dye to 10,000 parts of the medium. Malachite green inhibited the growth of the bacteria in a concentration of 1 to 100,000. The cultures were found to take up the colors of the dyes to a considerable degree. Because of this it was decided that no dye should be incorporated in the selective medium, for it was thought that the dye might obscure the characteristic yellow color of the wilt bacteria.

COMPOSITION OF THE SELECTIVE MEDIUM

The composition of the selective medium was determined on the basis of the results of the experiments just reported. The substances composing the medium were glycerol, 30 cc; ferric ammonium citrate, 10 g; sodium taurocholate, 3 g; sodium chloride, 15 g; sodium sulphate, 2.5 g; potassium phosphate (dibasic), 2.5 g; calcium chloride, 0.1 g; magnesium sulphate, 0.1 g; agar, 17 g; and distilled water to make a liter. The hydrogen-ion concentration of this medium was adjusted to pH 7.0.

The medium was prepared as follows: The agar was dissolved in the steamer in 400 cc of distilled water. The salts, with the exception of sodium taurocholate, were dissolved in a single container in 300 cc of water by gentle heating. Sodium taurocholate was dissolved in 200 cc of distilled water in the steamer. Following this, all the salt solutions, after the addition of the glycerol, were mixed with the melted agar and the liquid made up to a liter by the addition of distilled water. The medium was adjusted to pH 7.0 by the addition of approximately 17 cc of N/1 NaOH, tubed, and sterilized in the autoclave for 25 minutes at 15 pounds pressure.

EFFICIENCY OF THE SELECTIVE MEDIUM

The efficiency of the selective medium was determined by comparison with nutrient dextrose agar. Suspensions of wilt bacteria, soil, and a mixture of both were employed. The nutrient dextrose agar medium was selected as the standard of comparison because, of all the other common media tried, it was found to be most favorable for the growth of the wilt bacteria.

The relative efficiency of the two media was determined in the usual way by colony counts on comparable dilution plates. Colonies in all the plates appeared at the end of the third day after plating. The colonies on the nutrient agar plates were larger in diameter than those on the selective medium. However, the colonies on the selective medium were more raised. They were conspicuous owing to a more intense yellow color and a glistening appearance. The final counts of the plates were taken after an incubation period of 10 days,

by means of Frost's plate counter. The results showed that the number of colonies growing in the selective medium was almost twice as large as that of the colonies growing in the nutrient dextrose agar plates. In the case of the selective medium approximately one half of the colonies grew on the surface of the medium.

The influence of the selective medium on the soil micro-organisms that commonly appeared on the nutrient dextrose agar plates when attempts were made to isolate the wilt bacteria from the soil was tested in the following manner: Six soil suspensions of various concentrations were made. The heaviest of these suspensions was prepared by shaking thoroughly 50 g of rich greenhouse soil in a flask containing 500 cc of sterile water. The soil dilutions employed were 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} . One cubic centimeter of each suspension was placed in each of five Petri dishes by means of a pipette. The rest of the procedure was similar to that employed in the experiments described in the preceding paragraph. After an incubation period of 3 days at room temperature the plates of the first four suspensions containing nutrient agar were completely overgrown with bacteria and fungi; plates of the fifth suspension containing the same medium had an average of 200 colonies per plate. The plates of the suspensions in which the selective medium was employed had 2,000, 400, 40, 10, and 0 colonies, respectively. These results represent the average of three tests made at different times. From these results it appears that the selective medium is very unfavorable for the growth of a considerable number of soil bacteria and fungi.

When a certain number of the wilt bacteria were introduced in soil suspensions of various concentrations, the pathogene was recovered by means of the selective medium from some suspensions, but it was not recovered from the same suspensions when nutrient dextrose agar was used. The method of procedure in these tests was similar to that just described except that to each of the six soil suspensions an equal amount of a standardized suspension of Stewart's wilt bacteria was added. After an incubation period of 5 days about 5,000 colonies of the wilt bacteria appeared on the plates containing the selective medium of the third, fourth, fifth, and sixth suspension. The plates containing the nutrient agar, with the exception of these of the sixth suspension, were covered with various fungi and bacteria other than the Stewart's wilt pathogene.

The wilt bacteria were recovered from artificially infested soil a few days after their introduction, by using the selective medium. This was done in the following manner: Two boxes of soil were artificially infested with the wilt bacteria. One day after the treatment 50-g samples from each box were taken and suspended in 500 cc of sterile distilled water. The suspensions were shaken violently for 5 minutes. After the large soil particles had settled, part of each suspension was decanted and further diluted 100 times. Portions of 1 cc of each such suspension were placed in several Petri dishes and the melted medium poured in them. For checks, a similar set of plates was poured in which nutrient dextrose agar was used. The dishes were incubated at 28° C. and examined after 24 hours. The check plates were covered with bacterial and fungus growth, while the plates in which the selective medium was used were apparently free from any growth. On the fourth day after plating, numerous yellowish colonies

were observed on some of the selective-medium plates. It was later found that the colonies were mainly of three types, creamy white, yellow with orange-colored centers, and glistening buff colored, which in subsequent cultural and pathogenicity tests were found to be those of Stewart's wilt organism. The experiment was repeated by taking another sample of soil from each of the same boxes 5 days after its infestation. The bacteria were again recovered, although in smaller numbers, from the soil by using the selective medium. They did not appear on the plates in which nutrient dextrose agar was used.

Similar tests were made in which other bacterial wilt-producing cultures (3, 5) were used. These bacteria showed no evidence of growth on the selective medium agar plates. Nor did they show growth in test tubes with liquid selective medium and Uschinsky's solution 3 weeks after they were transferred, although a typical culture of *Phytomonas stewarti* grew in these media profusely. It therefore appears that the selective medium cannot be used to advantage with all wilt-producing bacteria.

SUMMARY

Symptoms of Stewart's wilt disease of corn caused by *Phytomonas stewarti* (Smith) Com.S.A.B. are described for both sweet and field corn. The appearance of water-soaked stripes, which later turned yellow and light brown, along the veins after puncture inoculations of the roots, stems, and leaves of young plants was found to be characteristic of this disease. Bacterial exudate upon the surface of diseased leaves and glumes was observed.

The life history of the causal organism in relation to pathogenesis was given particular attention.

The bacteria were found to enter the host tissue through bruised and wounded roots in artificially infested soil, and through wounds made by white grubs, the larval stage of *Phyllophaga* sp., feeding upon the roots in infested soil. The bacteria were not found to enter through the broken pericarp at the time of germination nor through unwounded roots.

In the leaves the bacteria were found in the vessels of the vascular bundles, in large cavities adjoining disrupted bacterium-filled vessels, in the intercellular spaces and substomatal cavities of the parenchymatous tissue, and between cells in the place of the dissolved middle lamellae. The presence and the activities of the bacteria in these tissues caused discoloration, plasmolysis, and death.

In the husks the location of the bacteria was found to be similar to that in the leaves.

In the stem, shank, and cob the bacteria were found in the vessels, in the large air spaces of some of the bundles, in masses in the disrupted pith tissue, and as exudate upon the surface of the shank.

In the tassel the bacteria were located in the vascular system of the rachises, rachillas, and the glumes, in the delicate spiral vessels of the filaments, and in the parenchymatous tissue of the glumes. The bacteria were isolated from the anthers and pollen of diseased plants.

In the kernel the bacteria were located in the vascular system of the chalazal region, in cavities adjoining ruptured spiral vessels, between the outermost layer of the chalazal region and the aleurone layer, and between the cells of the endospermic tissue.

The wilt organism was isolated from exudate of diseased leaves, shanks, husks, glumes, and kernels, from the water which accumulated between the blade and the ligule, and at the base of young uncurling leaves. By employing a selective medium the bacteria were isolated from overwintered corn stubble, from decaying roots and stems, and from disintegrating parts of diseased kernels after the germination of the kernels in the soil.

The transmission of the bacteria to a new host through diseased seed took place when an avenue of entrance for the bacteria was provided by injuring the roots of the young seedlings. The corn rootworm, the larval stage of *Diabrotica longicornis* (Say), transmitted the disease from diseased to healthy plants.

A selective medium was developed with which the Stewart's wilt bacteria were recovered from badly contaminated plant parts and from artificially infested soil. This medium was developed in connection with studies on the physiology of the causal bacteria.

Other bacterial cultures, apparently different from the typical Stewart's wilt organism in some cultural and physiological characters, when inoculated into corn plants produced symptoms more or less resembling those of Stewart's wilt. The growth of certain of these cultures was not favored by the selective medium.

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INHERITANCE OF CHARACTERS IN RICE¹

By JENKIN W. JONES

Senior agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

This paper deals with studies on the inheritance of the following characters in rice: Length of glumes, absence of ligules, brittleness, shattering, dwarfness, awns, color of awns, color of glumes, color of apiculi, and earliness.

LENGTH OF GLUMES

Most varieties of rice (*Oryza sativa* L.) have short (outer) glumes. The glumes usually vary in length from one fifth to one third of that of the lemma and palea. However, in some varieties the glumes are nearly as long, or quite as long, as the lemma and palea. These long-glumed varieties are reported to be resistant to lodging and shattering. They are not grown on a commercial scale in the United States.

Parnell et al. (9, pt. 1)² and Kato and Van der Stok, according to Ikeno (4), obtained in F_2 populations plants with short and long glumes in a ratio of 3:1. Chao (2), in a cross between a glutinous long-glumed variety and a common short-glumed variety, obtained in F_2 a ratio of 15 plants with short to 1 plant with long glumes. In this case short glumes were due to dominant duplicate genetic factors. Ramiiah et al. (10), in a cross between short- and long-glumed varieties, obtained F_1 plants that had glumes intermediate in length between the parents. In the F_2 populations the parental and intermediate glume lengths were obtained. These investigators placed the F_2 plants with respect to glume length in three groups, namely, short, intermediate, and long, and by this arbitrary grouping they obtained approximately a 1:2:1 ratio. However, there was much variation in the length of the glumes of plants classified as intermediate. In this group the glumes varied in length from almost as short as the short-glumed parent to nearly as long as the long-glumed parent. This indicates that the plants classed as intermediate for glume length were not genetically similar and suggests that the difference between the short-glumed and the long-glumed varieties probably was due to multiple factors.

In 1926, Yosemite, a normally short-glumed variety, was crossed with "Nimai Kawa Mochi" and "Weitin," long-glumed varieties. The parents and F_1 plants were grown in 1927. The spikelets of F_1 plants from both crosses had normal, short glumes.

In the F_2 population of the cross Yosemite $\times$ Nimai Kawa Mochi 1,116 plants were grown to maturity. Of these, 831 plants had

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² Reference is made by number (italic) to Literature Cited, p. 782.

spikelets with short glumes and 285 plants had spikelets with long glumes. These numbers agree with a monohybrid 3:1 ratio, the deviation being only 6.00 ± 9.76 plants. Of the 1,047 Yosemite $\times$ Weitin F_2 plants 785 had short glumes and 262 had long glumes, the deviation from a 3:1 ratio being 0.25 ± 9.46 plants. In the total F_2 population of the two crosses there were 1,616 plants with short glumes and 547 with long glumes, the deviation from a monohybrid ratio being 6.25 ± 13.59 plants.

Fifteen random F_2 progenies from the cross Yosemite $\times$ Nimai Kawa Mochi and 16 progenies from the cross Yosemite $\times$ Weitin were grown in the F_3 . Of the 15 progenies, 7 segregated for short and long glumes, 3 bred true for short glumes, and 5 bred true for long glumes. Of the 16 progenies, 7 segregated for short and long glumes, 4 bred true for short glumes, and 5 bred true for long glumes. The results for both groups are shown in table 1.

TABLE 1.—Breeding behavior of F_2 progenies from the crosses Yosemite $\times$ Nimai Kawa Mochi and Yosemite $\times$ Weitin grown in F_3 at the Biggs Rice Field Station, Biggs, Calif., 1929

Cross	Number of progenies	Number of plants with glumes		Deviation from 3:1 ratio and probable error
		Short	Long	
Yosemite $\times$ Nimai Kawa Mochi	3	605	—	3 25 $\pm$ 10 10
	7	901	297	
	5	—	837	
Yosemite $\times$ Weitin	4	704	—	18 50 $\pm$ 10 54
	7	961	346	
	5	—	765	
Total for 14 segregating progenies	—	1,868	643	15 25 $\pm$ 14 63

In the seven segregating progenies of each cross producing plants with short and long glumes the deviations from a 3:1 ratio were less than twice the probable error in all progenies, except for one in each cross. The results show that short glumes are dominant to long glumes and that the long-glumed and short-glumed varieties used in this study differ by a single genetic factor for this character.

ABSENCE OF LIGULES

In commercial rice varieties the ligules at the juncture of the leaf blade and sheath are usually well developed. A liguleless variety, "Tsutsuito," the only one known to the writer, was obtained in Japan in 1925. Tsutsuito is late-maturing, liguleless, and awnless. It was crossed with "Kotake," also late-maturing, and partly awned.

The F_1 plants had normal ligules and were partly awned like the male parent Kotake. An F_2 population, consisting of 1,024 plants, was grown in 1927. Of this number 796 plants had normal ligules and 228 were liguleless, the deviation from a 3:1 ratio being 28.00 ± 9.35 plants.

Fifteen random F_2 progenies were grown in the F_3 in 1928, of which 6, consisting of 989 plants, bred true for normal ligules, 5 segregated

and gave 655 plants with ligules to 195 without ligules, and 4, consisting of 725 plants, bred true for the liguleless character.

The five progenies that segregated in F_3 produced 655 plants with and 195 plants without ligules, the deviation from a 3:1 ratio being 17.50 ± 8.51 plants. According to Ikeno (4), Kato found that normal ligules were dominant to the liguleless character in rice. He obtained in F_2 3 plants with normal ligules to 1 without ligules.

BRITTLINESS

The culms and leaves of commercial rice varieties vary in strength and toughness. Under normal conditions some varieties do not lodge, whereas others lodge badly. Between these two extremes there are various degrees of resistance to lodging. However, all the commercial varieties are normal in that the straw possesses a certain degree of toughness or tensile strength and is somewhat resistant to complete breaking by bending or twisting. In contrast to the normal toughness of the culms and leaves of the commercial varieties is the extreme brittleness of the culms, leaves, and other parts of "Kama Irazu," a variety obtained in Japan. This variety does not lodge, although the stalks bend over under the weight of heavy panicles. However, in both the green and the mature stages the leaves and culms are so brittle that they break off at the slightest pressure. The writer was informed that the name Kama Irazu means "no need of a sickle in harvesting." This variety was so named because a standing plant gathered into the hand snaps off when given a slight twist. The panicles also are brittle and the spikelets fall off easily. In threshing, the lemma and palea often are removed and the kernel is easily broken.

In the cross Kama Irazu $\times$ Colusa (C.I.³ 1600, a normal variety) the F_1 plants had normal (not brittle) stalks, leaves, and panicles. In the second generation there were 479 plants with normal stalks, leaves, and panicles to 156 plants with these parts brittle. The deviation from a 3:1 ratio was 2.75 ± 7.36 plants. The segregation into normal and brittle plants was very distinct. The ordinary green rice leaf often can be folded up without breaking into separate parts, but the brittle leaf is easily snapped off when bent. Owing to this fact it was possible to classify the plants even before they headed.

Twenty-five random F_2 progenies were grown in the F_3 . Six of these, consisting of 292 plants, bred true for normal plants; 14 segregated, producing 453 normal plants to 142 brittle ones; and 5, consisting of 223 plants, bred true for the brittle character. The segregation of each of the 14 progenies agreed well with a ratio of 3 normal plants to 1 brittle plant, the deviation for the 14 progenies being 6.75 ± 7.12 plants.

Analyses were made of the straw and grain of Kama Irazu and of Colusa (C.I. 1600).⁴ The straw of Kama Irazu contained less ash, silica, and crude fiber than that of Colusa, but a higher percentage of lignin, nitrogen, and moisture. The chemical data do not show the cause of brittleness in Kama Irazu, but this character may be due to cell structure or arrangement.

³ C.I. denotes accession number of the Division of Cereal Crops and Diseases.

⁴ These analyses were made by Dr. Jehiel Davidson, of the Bureau of Chemistry and Soils, U.S. Department of Agriculture.

SHATTERING

Varieties of rice differ greatly with respect to the tightness with which the grain is held on the panicle before and after maturity. In certain varieties the grain is held so tight that threshing is difficult. In others the grain shatters very easily, even when carefully handled. Between these two extremes there probably are varieties representing all degrees of tightness.

In the United States commercially grown rice varieties are more or less resistant to shattering but do not hold the grain so firmly that they cannot be satisfactorily threshed with modern machinery. Varieties that shatter too easily are not suited for harvesting with grain binders. In countries where rice is harvested by hand and threshed by treading and flailing, varieties that shatter rather easily are grown in preference to those that shatter less readily.

Takenouchi (12) reports that at the juncture of the rice grains and their supporting stalks there is a special tissue consisting of from 1 to 3 layers of lignified thin-walled cells. In rices that shatter easily these cells develop and dry up early, whereas in rices that shatter less easily they do not dry up so early.

According to Matsuura (6), Kato crossed a nonshattering rice with one that shattered easily. In the F_1 the plants were nonshattering, and in the F_2 the ratio of nonshattering plants to those that shattered easily was 3:1.

The Caloro and Colusa varieties, which are extensively grown in California, do not shatter easily if properly handled. These were crossed with Chinese varieties that shattered easily. The crosses were C.I. 7075 $\times$ Colusa, C.I. 7078 $\times$ Caloro, and C.I. 7389 $\times$ Caloro.

The F_1 plants of these crosses appeared to be intermediate. They did not shatter so readily as did the Chinese varieties, and yet they did not seem to hold the grain quite so firmly as did Colusa and Caloro.

Owing to various degrees of sterility in the F_2 plants of these crosses and to the failure of many individual plants to mature grain, only part of the F_2 population was suitable for a study of the inheritance of shattering.

On the basis of the ease or difficulty of stripping the grain from the mature panicles of plants standing in the field, it was not hard to determine relative resistance to shattering. Only a few trials were necessary to reduce this determination to a fair degree of accuracy. The plants from which it was easy to remove the seed were classed as easily shattered, whereas those from which it was rather difficult to remove the seed were classed as nonshattering.

In the F_2 population from the cross C.I. 7075 $\times$ Colusa there were 29 nonshattering plants to 11 that shattered readily; from the cross C.I. 7078 $\times$ Caloro there were 99 nonshattering plants and 46 that shattered readily; and from the cross C.I. 7389 $\times$ Caloro there were 249 nonshattering plants and 109 that shattered readily. In each cross these numbers agree reasonably well with a 3:1 ratio. The deviations from this ratio in the order given were 1.00 ± 1.85 plants, 9.75 ± 3.52 plants, and 19.5 ± 5.53 plants. The results indicate that in the varieties used in this study the nonshattering and shattering characters probably differ by a single genetic factor.

DWARFNESS

Dwarf varieties of rice often are grown at rice experiment stations. While they are of no economic importance, they are of interest to plant breeders. According to Ikeno (4), a dwarf rice plant was described by Iwasaki in 1828.

Parnell et al. (9, pt. 2) first reported on studies of the inheritance of dwarfness in rice. They found that dwarfness is a simple recessive to normal. The average height of the normal parent used in their study was 49.7 inches and that of the dwarf parent 27.9 inches. The average height of the normal segregates in F_2 was 50.5 inches and of the dwarf segregates 31.2 inches.

Sugimoto (11) states that dwarfness usually is a simple recessive to normal. He found a dwarf mutant in a true-breeding normal variety, Waseshinriki, which was a simple recessive to normal. Another dwarf appeared as a mutant in a true-breeding strain isolated from a hybrid. This dwarf was dominant to normal and in the F_2 segregation produced 3 dwarf plants to 1 normal.

Akemi (1) crossed the normal variety Akage with the dwarf varieties "Daikoku" and "Ebisu." In the F_2 segregation both crosses produced 5 normal plants to 1 dwarf. In a cross between the dwarf varieties Daikoku $\times$ Ebisu the F_1 was normal, and the segregation in F_2 gave 9 normal to 3 Daikoku to 3 Ebisu to 1 new very short dwarf type, Kodaikoku. Akemi assumed that the genetic constitution of normal was $AABB$, Ebisu $AAbb$, Daikoku $aaBB$, and Kodaikoku $aabb$.

Nagai (8) reported a dwarf mutant that was recessive to normal. This mutant showed a peculiar mode of inheritance in that in successive generations mutant individuals gradually increased in proportion to normals.

The writer obtained in Japan seed of a dwarf variety known as "Daikokunc." This variety has short, rather wide, erect leaves, short culms, and a short, compact panicle that looks more like a spike of wheat than a panicle of rice. It is partly awned and the kernel is short and round and enclosed by a rather coarse hull. The cross Yosemite $\times$ Daikokunc was made in 1927. Yosemite is an early-maturing, awnless variety of normal height.

The F_1 plants were intermediate in maturity, partly awned, normal in appearance, and somewhat taller than the normal parent. The average height of the normal parent was 38 inches, of the dwarf parent 14 inches, and of the F_1 plants 43 inches. The average length of 10 panicles of the normal variety was 4.7 inches, of the dwarf parent 2.3 inches, and of the F_1 plants 4.6 inches.

The F_2 population consisted of 677 plants that were grown to maturity, of which 547 were normal and 130 were dwarf. Segregation into the two groups was very distinct. The deviation from a 3:1 ratio was 39.25 ± 7.60 plants, or more than five times the probable error, whereas the deviation from a 13:3 ratio was only 3.0625 ± 6.85 plants. However, the segregation of random F_2 progenies grown in F_3 shows that the 3:1 ratio is correct.

In the F_2 population the average height of 100 normal segregates was 37.4 inches and the average height of 100 dwarf segregates was 16.9 inches. The average height of 42 plants of the normal variety was 32.6 inches and the average height of 42 dwarf plants was 13.9 inches. The normal and dwarf segregates in F_2 exceeded the normal and dwarf parent varieties in average height.

Of 25 random F_2 progenies grown in 1930, 7, consisting of 367 plants, bred true for normal stature, 15 segregated for normal and dwarf plants, and 3, consisting of 86 plants, bred true for dwarf plants. Each of the 15 segregating progenies produced normal and dwarf plants in numbers that agreed well with a 3:1 ratio. The total for the 15 progenies was 598 normal to 181 dwarf plants, the deviation from a 3:1 ratio being 13.75 ± 8.15 plants. In the varieties used in this study dwarf character was a simple recessive to normal.

AWNS

Rice varieties may be classed as fully awned, partly awned, and awnless. In fully awned varieties all spikelets are awned but the awns often vary in length. In partly awned varieties awned and awnless spikelets are present on the same panicle. In true awnless varieties the awns are absent and do not develop under any conditions. In some varieties the main panicle may be awnless, whereas some of the spikelets on branch culms and later tillers may be partly awned. The extent of development of the awns in rice is controlled by climatic conditions and soil fertility as well as by genetic factors. In some crosses it is difficult to separate the awn types because climatic conditions and soil fertility have such a marked influence upon their development.

Chao (2), Hoshino (3), Jones (5), Mendiola (7), Nagai (8), Yamaguchi (13), and Kato and Van der Stok, according to Matsuura (6), have reported that in F_1 awns are dominant or partially dominant to the awnless character in rice. A segregation in F_2 of 3 awned or partly awned plants to 1 awnless plant has been reported by Jones (5), Nagai (8), and Chao (2), and a ratio of 9 fully awned to 6 partly awned to 1 awnless plant by Jones (5). The awned group in the 15:1 ratio reported by Chao (2) were designated as fully awned, mostly awned, and rarely awned plants.

In the few crosses between fully awned and awnless varieties that have been studied by the writer, three groups of awned plants have been observed in the F_2 segregations. These are fully awned plants, partly awned plants with awns about the same length as in fully awned plants but present only on part of the spikelets on a panicle, and partly awned plants with short awns often confined to the spikelets near the tip of the panicles. The plants in this last group may be referred to as tip-awned. It appears that fully awned varieties often differ from awnless varieties by two independent genetic factors. If this is true, the fully awned group from a cross between a fully awned and an awnless variety should differ from the partly awned and tip-awned groups by a single genetic factor, and the partly awned group should differ from the tip-awned group by two genetic factors. Data in support of this view are reported herein.

AWNLESS $\times$ FULLY AWNED

In the cross Colusa (awnless) $\times$ Aikoku (fully awned) the F_1 plants were partly awned. In the F_2 segregation 607 plants were classed as fully awned, partly awned, and tip-awned to 71 awnless plants. The deviation from a 15:1 ratio in this case is 28.63 ± 4.25 plants. This deviation is very large.

Twenty-five random F_2 progenies were grown in F_3 . Five progenies produced 225 fully, partly, and tip-awned plants to 13 awnless

plants. These numbers agree with those expected in a 15 : 1 ratio, the deviation being 1.88 ± 2.52 plants. Six progenies, consisting of 309 plants, produced fully awned, partly awned, and tip-awned plants in what appeared to be a ratio of 3 fully awned to 1 partly awned, and 3 partly awned plants to 1 tip-awned. However, the awn types in this cross could not be separated with certainty. Ten progenies produced no fully awned plants, but 347 partly awned and tip-awned plants to 120 awnless plants, deviating from a 3 : 1 ratio by 3.25 ± 6.31 plants. One progeny, consisting of 46 plants, appeared to be fully awned, and three progenies, consisting of 119 plants, appeared to produce only partly awned or tip-awned plants. The number of random F_2 progenies was too small to yield all the possible segregations and true-breeding groups that were expected. However, the results suggest that the fully awned variety Aikoku probably differs from the awnless variety Colusa by two main genetic factors for awns.

PARTLY AWNED $\times$ FULLY AWNED

In the cross Caloro (partly awned) $\times$ Butte (fully awned) the F_1 plants were fully awned. The segregation for awns was not studied in F_2 , but a study was made of the segregation of 11 random F_2 progenies grown in F_3 . Each of the 11 F_2 progenies and the total of the 11 progenies produced fully awned and partly awned plants in a ratio of 3 : 1. In the 11 F_2 progenies there were 393 fully awned to 99 partly awned plants, the deviation from a 3 : 1 ratio being 24.00 ± 6.48 plants. This deviation, which is more than three times the probable error, was probably due to errors in classifying the plants resulting from the natural variability of awn development. In the individual progenies there were 3 small plus deviations, 7 minus deviations, and 1 progeny with no deviation from the 3 : 1 ratio.

PARTLY AWNED $\times$ AWNLESS

Colusa, Chinese Originario, Maratelli, and Yosemite, awnless varieties, were used as female parents in crosses with Wataribune and Caloro, partly awned varieties. Yosemite, Chinese Originario, and Colusa were crossed with Wataribune, Yosemite, and Chinese Originario, and Maratelli was crossed with Caloro. In each of the six crosses the F_1 plants were partly awned.

TABLE 2.— F_2 segregation of crosses between awnless and partly awned rice varieties grown at the Biggs Rice Field Station, Biggs, Calif., 1929

Cross		Number of F_2 plants			Deviation from 3 : 1 ratio and probable error
Female, awnless	Male, partly awned	Partly awned	Awnless	Total	
Yosemite.....	Wataribune.....	450	199	649	36.75 $\pm$ 7.44
Chinese Originario.....	do.....	421	121	542	14.50 $\pm$ 6.80
Colusa.....	do. ^a	251	94	345	7.75 $\pm$ 5.42
Chinese Originario.....	Caloro.....	473	161	634	2.50 $\pm$ 7.35
Yosemite.....	do.....	423	178	601	27.75 $\pm$ 7.16
Maratelli.....	do.....	484	184	668	17.00 $\pm$ 7.55

^a Grown in 1930.

The F_2 segregation of the six crosses is shown in table 2. In all crosses the number of partly awned and awnless plants agrees well with a 3 : 1 ratio, except for the crosses Yosemite $\times$ Wataribune and

Yosemite $\times$ Caloro. The deviations in these crosses are large and suggest the possibility of a modifying factor in the Yosemite variety.

Twenty-five random F_2 progenies from the crosses Yosemite $\times$ Wataribune, Chinese Originario $\times$ Wataribune, Colusa $\times$ Wataribune, and Chinese Originario $\times$ Caloro were grown in F_3 . The breeding behavior of these progenies is shown in table 3.

TABLE 3.—Breeding behavior in F_3 of F_2 progenies from crosses between awnless and partly awned rice varieties grown at the Biggs Rice Field Station, Biggs, Calif., 1930

Cross		Number of F_2 progenies grown in F_1	Number of F_3 plants		Deviation from 3:1 ratio and probable error
Female, awnless	Male, partly awned		Partly awned	Awnless	
Yosemite	Wataribune	3 16 4	142 605	153 197	36.5 $\pm$ 8.04
Chinese Originario	do	6 13 4	258 494	136 170	
Colusa	do	7 11 7	349 446	168 360	14.50 $\pm$ 7.24
Chinese Originario	Caloro	11 11 2	550 429	121 100	

^a 2 F_2 progenies did not agree with 3:1 ratio in F_3 in these 2 crosses

^b Grown in 1931

^c 1 spikelet on each of 4 panicles appeared to have a tip awn

^d 1 F_2 progeny did not agree with 3:1 ratio in F_3

In each cross three or more F_2 progenies bred true for partly awned plants and two or more bred true for awnless plants. Of the F_2 progenies that segregated in F_3 all produced partly awned and awnless plants in a ratio of 3:1, except 1 or 2 progenies in each cross. The failure of some segregating F_2 progenies to give the expected 3:1 ratio may have been due to errors in classification or possibly to natural crossing in F_1 , as the F_1 plants were not bagged. The fact that nearly all the F_2 progenies gave the expected 3:1 ratio in F_3 indicates that the partly awned and awnless varieties used, with the possible exception of Yosemite, probably differ by a single genetic factor.

The data on the inheritance of awns in crosses between an awnless and a fully awned variety, a partly awned and a fully awned variety, and awnless and partly awned varieties indicate that the fully awned varieties differ from Colusa, awnless, by two independent genetic factors. Butte, fully awned, appears to differ from Caloro, partly awned, by a single genetic factor, and Caloro and Wataribune, partly awned, appear to differ from the awnless varieties by a single genetic factor.

COLOR OF AWNS, GLUMES, AND APICULI

AWNS

Aikoku has red awns, glumes, and apiculi, whereas Colusa is awnless and has green apiculi and glumes, which are straw-colored at maturity. In the cross Colusa $\times$ Aikoku the F_1 plants had red awns, glumes, and apiculi. In the F_2 population 454 plants had red awns and 153 plants had green awns. These numbers agree well with a 3:1 ratio, the deviation being only 1.25 $\pm$ 7.20 plants.

Of 25 random F_2 progenies grown in F_3 , 4, consisting of 185 plants, bred true for red awns; 13 produced 392 plants with red awns to 126 plants with green awns; 3 progenies, consisting of 133 plants, bred true for green awns; and of 5 remaining F_2 progenies, 3, consisting of 141 plants, segregated for red-awned and awnless plants with red glumes and apiculi, and 2, consisting of 97 plants, segregated for green-awned and awnless plants with green glumes and apiculi. Each of the 13 F_2 progenies that segregated for red and green awns did so in a ratio of 3:1. In the 13 progenies as a group the deviation from a 3:1 ratio was 3.50 ± 6.65 plants, which is a satisfactory fit.

Caloro is partly awned and has green awns and glumes. In the cross Caloro $\times$ Aikoku the F_1 plants had red awns and glumes. In the F_2 population there were 402 plants with red awns to 261 plants with green awns. These numbers are in fair agreement with a 9:7 ratio. The deviation was 29.0625 ± 8.62 plants. Of the F_2 population, 663 plants were classed as awned and 15 as awnless. One random F_2 progeny, which was classed as awnless in F_2 , was partly awned in F_3 . Therefore, it is probable that other plants classed as awnless in F_2 carried factors for awns but that owing to environmental conditions no awns were visible.

Of the random F_2 progenies grown in F_3 , 4, consisting of 202 plants, bred true for red awns; 5 produced 143 plants with red awns to 105 plants with green awns; 9 produced 269 plants with red awns to 90 plants with green awns; and 7, consisting of 301 plants, bred true for green awns. The deviation from a 9:7 ratio for 5 of the F_2 progenies was 3.50 ± 5.27 plants, which is a satisfactory fit. The deviation from a 3:1 ratio for 9 of the F_2 progenies was 0.25 ± 5.53 plants, or almost a perfect fit.

Butte is a fully awned variety with red awns. In the cross Caloro $\times$ Butte the F_1 plants had red awns. In the segregation in F_2 , 271 plants had red awns to 251 plants with green awns. In this case the deviation from a 9:7 ratio is 22.625 ± 7.64 plants. Twenty random F_2 progenies were grown in F_3 . Four, consisting of 198 plants, bred true for red awns; 4 produced 91 plants with red awns to 63 plants with green awns, or in a ratio of 9:7; and 12 progenies, consisting of 560 plants, bred true for green awns.

In the cross Colusa $\times$ Aikoku the difference in awn color apparently was due to a single genetic factor, whereas in the crosses Caloro $\times$ Aikoku and Caloro $\times$ Butte the difference appeared to be due to two complementary genetic factors. The results indicated that Colusa has one complementary genetic factor in common with Aikoku and Butte (5). However, Caloro appears to differ in awn color from Butte and Aikoku by two complementary genetic factors.

GLUMES AND APICULI

Aikoku has red glumes and Colusa has green glumes. In the cross Colusa $\times$ Aikoku the F_1 plants had red glumes. Segregation for color of glumes in F_2 gave 504 plants with red glumes to 174 plants with green glumes. The deviation from a 3:1 ratio is 4.50 ± 7.60 plants.

Of the 25 random F_2 progenies grown in F_3 , 7, consisting of 326 plants, bred true for red glumes; 13 produced 460 plants with red to 163 plants with green glumes; and 5, consisting of 230 plants, bred true for green glumes. Each of the 13 F_2 progenies that segregated

for glume color produced plants with red and green glumes in a ratio of 3:1. The deviation from this ratio for the 13 F_3 progenies was 7.25 ± 7.29 plants.

In the cross Caloro $\times$ Aikoku, green by red glumes, the F_1 plants had red glumes. In F_2 the segregation for color of glumes gave 410 plants with red glumes to 268 with green glumes. The deviation in this cross from a 9:7 ratio was 28.63 ± 8.71 plants.

In the crosses Colusa $\times$ Aikoku and Caloro $\times$ Aikoku the color of the apiculi was always the same as that of the glumes and appeared to be controlled by the same genetic factor or factors.

The red glumes of Aikoku differ from the green glumes of Colusa by a single genetic factor and from the green glumes of Caloro by two dominant complementary genetic factors. The same is true of the color of the apiculi of these varieties. In all hybrids studied the color of the apiculi of the lemma and palea was the same as that of the awns. However, the writer knows of one variety with red apiculi, the awns of which are green at maturity.

COLOR OF LEMMA AND PALEA FURROWS

The cross Yosemite $\times$ Weitin, used in the study of glume length, also presented an opportunity to study lemma and palea furrow color. In Weitin the lemma and palea furrows are yellowish brown, and in Yosemite they are straw-colored at maturity. In F_1 plants of the cross Yosemite $\times$ Weitin the furrows were yellowish brown. Of 1,047 F_2 plants 834 had yellowish-brown and 213 had straw-colored lemma and palea furrows. These numbers agree well with a 13:3 ratio, with yellowish brown dominant to straw color, the deviation from the 13:3 ratio being 16.69 ± 8.52 plants.

Segregating F_3 progenies were not classified for color of the lemma and palea furrows. It was observed, however, that the 7 F_2 progenies segregating for short and long glumes also segregated for color of lemma and palea furrows. Three of the 4 F_3 progenies that bred true for short glumes had yellowish-brown lemma and palea furrows, and the other family segregated for furrow color. One of the 5 F_3 progenies that bred true for long glumes had yellowish-brown lemma and palea furrows, 1 progeny segregated for furrow color, and 3 progenies bred true for long glumes with straw-colored lemma and palea furrows.

The factors for yellowish-brown color of the lemma and palea furrows and the factor for short glumes appear to be inherited independently, as shown in table 4.

TABLE 4.—*Phenotypes and observed and calculated number of F_2 plants in each group from the cross Yosemite $\times$ Weitin, grown at the Biggs Rice Field Station, Biggs, Calif., 1928*

Phenotype ^a	Observed	Calculated	$\frac{(O-C)^2}{C}$
Short glumes, yellowish-brown lemma and palea furrows.....	637	638.04	0
Short glumes, straw-colored lemma and palea furrows.....	148	147.24	0
Long glumes, yellowish-brown lemma and palea furrows.....	197	212.68	1.16
Long glumes, straw-colored lemma and palea furrows.....	65	49.08	5.16
	1,047	1,047.04	---

$P=0.0089$ $\chi^2=6.32$.

^a Expected ratio 39:9:13:3.

EARLINESS

In the cross Colusa (an early variety) $\times$ Wataribune (a late variety) the F_1 plants and Wataribune matured on October 4 and Colusa on September 19. In the F_2 population there appeared two quite distinct groups with respect to maturity, namely, early and late. On September 6 the early plants were fully headed and starting to mature whereas the late plants were just coming into full heading. On this date the F_2 plants were classified as early and late on the basis of stage of development. There were 264 late to 81 early plants, the deviation from a 3:1 ratio being 5.25 ± 5.42 plants.

Twenty-five random F_2 progenies were grown in F_3 . Six progenies, consisting of 232 plants, bred true for late maturity; 12 progenies that segregated for late and early plants produced 485 late to 186 early maturing plants; and 7 progenies, consisting of 341 plants, bred true for early maturity. The deviation from a 3:1 ratio for the 12 segregating progenies was 18.25 ± 7.57 plants.

Other studies and observations on the inheritance of earliness in rice have indicated that multiple factors often are involved. It is of interest, therefore, to note the simple segregation in this case. The factorial situation for this as well as for other characters in rice varies with the varieties used.

SUMMARY

The rice crosses used in these studies were grown for three generations, except those used in a study of shattering.

In the varieties studied short glumes were dominant to long glumes.

The characters normal ligule, normal culm strength, and nonshattering were found to be simple dominants to absence of ligules, brittleness of culm, and shattering of grain, respectively.

The dwarf variety studied was recessive to the normal.

In the crosses awnless $\times$ fully awned, partly awned $\times$ fully awned, and partly awned $\times$ awnless varieties, the data indicate that the fully awned varieties used differ from awnless varieties by two genetic factors, fully awned varieties differ from partly awned by a single genetic factor, and partly awned varieties differ from awnless varieties by a single dominant genetic factor.

In these studies red color in the awns, glumes, and apiculi was dominant to green (straw color). Ratios of 3:1 and 9:7 were obtained, which show that the color was due to a single genetic factor and to two complementary genetic factors.

In Weitin brownish-yellow lemma and palea furrows appeared to be inherited independently of long glumes. The brownish-yellow color of lemma and palea furrows was dominant to straw color and appeared to be due to two genetic factors resulting in a 13:3 ratio.

Plants from the cross Colusa $\times$ Wataribune were obtained in the ratio of 3 late to 1 early.

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IDENTITY AND HOST RELATIONS OF THE ELSINOE OF LIMA BEAN¹

By S. C. BRUNER, chief, Department of Entomology and Plant Pathology, Agricultural Experiment Station, Santiago de las Vegas, Cuba, and ANNA E. JENKINS, associate pathologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

This paper presents the results of inoculation tests on legumes with the fungus causing scab of the lima bean (*Phaseolus lunatus macrocarpus* Benth.). This organism was tentatively identified² as *Elsinoe canarialiae* Rac., causing scab of *Canaria* in the Orient. The plants inoculated were wild or naturalized lima bean, known in Cuba as "frijol caballero"; several common horticultural varieties of lima bean, including Fordhook Bush, Henderson Bush, Henderson Pole, and Challenger Pole; and some other leguminous plants, namely, *Calopogonium caeruleum* (Benth.) Hemsl., *Canaria gladiata* (Jacq.) DC., *C. ensiformis* (L.) DC., *Dolichos lablab* L., *Phaseolus vulgaris* L., *Pisum sativum* L., and *Stizolobium deeringianum* Bort. The object of the investigations was to obtain additional data on the host range of the organism, which would be of value taxonomically and otherwise, and also to determine some of the environmental conditions necessary for infection of the lima bean.

MATERIALS AND METHODS

The inoculations were made at the Agricultural Experiment Station, Santiago de las Vegas, Cuba, from 1930 to 1932 by the senior writer. The plants of *Calopogonium caeruleum* and some of those of *Canaria ensiformis* were inoculated on the station grounds where they were growing. The other inoculations were made in the plant-quarantine house. The sides of this house were protected with panels of fine copper-wire screening, which made it practically insect-proof but not fungus-proof. No infected lima-bean fields were located nearby, and no accidental infections took place on any of the plants under observation—either those inoculated or those used as controls. In fact, the conditions were such that there was no spread of the disease from the primary infections resulting from the artificial inoculations. There was no appreciable condensation of dew on the plants, and, moreover, they were largely protected from wind and rain—factors believed to greatly favor the dissemination of the fungus in the field.

The legumes inoculated were mostly grown from seeds sown in pots in a compartment of the plant-quarantine house not used for any other purpose. The culture of *Elsinoe* used (culture 254, A. E. Jenkins) was isolated at Washington, D.C., from cultivated green lima beans collected at Wajay, Cuba, November 5, 1929, by C. Aguiar, chief inspector of the Plant Inspection Service, Department of Agriculture, Commerce, and Labor, Cuba.

¹ Received for publication Aug. 8, 1933, issued January 1934.

² JENKINS, A. E. LIMA BEAN SCAB CAUSED BY ELSINOE. Jour. Agr. Research 42: 13-33, illus. 1931.

Most of the inoculations were made by the cotton-plaster method employed by Winston³ in inoculating *Citrus* with the closely related citrus-scab organism (*Sphaceloma fawcettii* Jenkins). This method consists of spreading small mycelial masses of the fungus grown on potato-dextrose agar on a piece of wet absorbent cotton and then applying this plaster to the uninjured plant surface. The leaf or other part inoculated, together with the cotton plaster, is then wrapped with waxed paper to prevent evaporation. In the present inoculations the paper was held in place by small paper clips, thread, or rubber bands, as was most convenient in each case. The plasters were removed after 24 hours, as preliminary inoculation tests had shown that the duration of the incubation stage did not exceed this period. It was not possible in all cases to arrange the wax paper so that the cotton

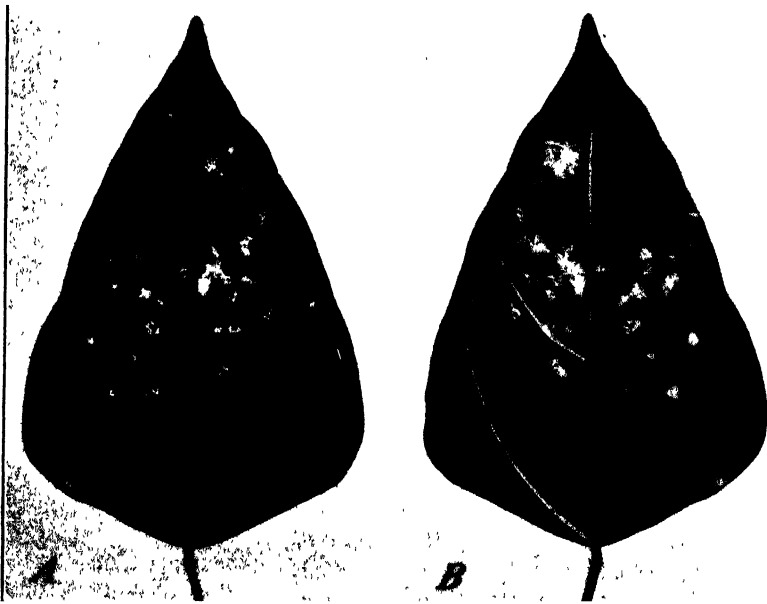


FIGURE 1—Leaflet of Henderson Bush lima bean 13 days after inoculation on upper surface with a culture of *Elasmoe*. A, Upper leaf surface, B, lower leaf surface $\times 4/5$ Santiago de las Vegas, Cuba, Apr. 7, 1930.

would remain moist for 24 hours without at the same time causing injury to the tender growth. Such injury occurred in a number of the inoculation tests that were omitted from table 1.

A few additional inoculations on cultivated lima beans were made by means of a medical atomizer, with which the plants were sprayed with a water infusion of a culture. Since the relative humidity of the quarantine house was somewhat below that prevailing outdoors, these plants dried quickly. Other plants were inoculated by the same method except that they were sprayed first with water alone and, after inoculation, were covered for 24 hours with a large bell jar in order to maintain as nearly as possible a saturated atmosphere during this period.

* WINSTON, J. R. CITRUS SCAB ITS CAUSE AND CONTROL. U.S. Dept. Agr. Bul. 1118, 39 pp., illus. 1923.

In all cases the cultures used as inocula were several weeks old and probably contained few or no conidia at the time they were transferred to the cotton plasters. Conidial formation may have taken place, however, soon after the culture was transferred from the test tube to the wet cotton. Conidial formation under similar conditions has already been described.⁴

INOCULATIONS ON LIMA BEANS

Table 1 gives the results of inoculations that were made on cultivated and on wild or naturalized lima beans by means of the cotton-plaster method. These data show that under suitable conditions the *Elsinoe* causing lima-bean scab readily infects the uninjured leaves and pods of the wild or naturalized lima bean and those of the common horticultural varieties listed. The data also show that infection occurs on tender leaves between 1.5 cm and 5.0 cm in length, the lesions usually becoming visible within 5 to 9 days. Where the upper leaf surface was inoculated the lesions were convex above and concave below; the reverse situation obtained when the lower leaf surface was inoculated. The leaflet shown in figure 1 was inoculated on the upper surface when 2 cm in length; it was photographed 13 days later. Typical scab lesions were obtained on tender pods from 1 to 3.5 cm long in about the same length of time as they were obtained on leaves. On a large, almost mature green pod, flat reddish lesions were produced, but actual infection may not have resulted in this older growth. Noninfection in the case of the tender stems and of a few of the inoculated leaves was possibly due to faulty technic or, as sometimes occurs, to the failure of conidia to form when a culture of the fungus is transferred to a humid environment.

As was to be expected of the plants inoculated by means of the medical atomizer, only those held under bell jars for 24 hours after inoculation became infected. Infection was evident within 7 days on several of the younger leaves, their leaflets then measuring from 4.5 cm to 11 cm in length. The checks remained healthy. The results of this experiment are consistent with observations made at Loiza, P.R., during the winter and spring of 1932.⁵ The disease was abundant there until the middle of February. During this period there was a great deal of rain. The disease almost disappeared from the plantings, however, during the 2 months of drought that followed, although it was present in other districts where the drought was less severe. The controls for all these inoculations on lima beans remained healthy.

⁴ JENKINS, A. E. (See footnote 2.)

⁵ Data obtained from the Bureau of Plant Quarantine, U.S. Department of Agriculture.

TABLE 1.—Results of inoculations on lima bean by the cotton-plaster method, 1930

Type or variety	Part inoculated	Length of leaflet or pod (cm)	Date inoculated	Result		Comment
				Positive	Negative	
Bush.	Tender leaflet, lower surface	2	July 15	+	—	Infection distinct in 8 days
	Large green pod	—	Aug. 1	+	—	Small reddish spots only in 11 days
	Tender pod	3	Aug. 7	+	—	Infection visible in 7 days, distinct in 9 days.
	do	2.5	do	+	—	Typical spots
	do	3.25	Aug. 12	+	—	Infection visible in 8 days, distinct in 11 days.
Fordhook Bush.	Tender leaflet, lower surface	3.8	Aug. 26	+	—	Infection distinct in 8 days
	Leaflet from same plant as the above one.	2	do	+	—	Infection faintly visible in 6 days, distinct in 8 days
	Tender leaflet, upper surface	3	Mar. 18	+	—	Spots distinct in 9 days
Henderson Bush.	Tender leaflet	1.5	Mar. 20	+	—	Convex above, Infection visible in 9 days
	Tender stem	3	do	—	—	
	Tender leaflets	5	do	—	—	
	do	2	Mar. 25	+	—	Infection distinct in 7 days
	Tender leaflet, upper surface	2.5	do	+	—	Do
	Tender leaflet, lower surface	2.5	do	+	—	
	Tender pods	{ 1 2 }	Apr. 9	—	—	{ Both withered and fell off, check normal
Challenger Pole	Flower spike with tender pod	1	do	+	—	Typical spots 16 days later on pods
	Tender leaflet, upper surface	3	Mar. 22	+	—	Spots distinct in 9 days
	End of tender shoots with unfolding leaves	—	do	—	—	
	Tender leaflet, upper surface	2.5	Mar. 27	+	—	Infection visible in 5 days
	Tender leaflet, lower surface	3	Aug. 30	+	—	Infection visible in 6 days, distinct in 8 days
	Tender leaflets, upper surface	1.75	Apr. 2	+	—	
Frijol caballero (pole type).	Tender leaflets	5	do	+	—	Infection visible in 7 days
	Tender leaflets, both surfaces.	4	Apr. 9	+	—	Infection distinct in 9 days
	Tender leaflet, lower surface.	2.5	July 15	—	—	
	do	4	Aug. 1	—	—	
	Tender leaflet	3.5	Aug. 7	+	—	Infection visible in 6 days, distinct in 7 days

INOCULATIONS ON OTHER LEGUMES

As previously stated, the cotton-plaster method was employed in all the inoculations on leguminous plants other than lima bean. The results were all negative (table 2). In addition to those reported in table 2, five tests were carefully made on *Canavalia gladiata* in the field on June 25, 1930, and on August 24, 1932, but no infection resulted. The tender leaflets ranged from 1.7 to 4.0 cm in length at the time of inoculation.

The uniformly negative results obtained from these inoculations are of particular interest in the case of *Canavalia*, for they are in accord with field observations made in Puerto Rico ⁶ and in Cuba. Both species of *Canavalia* listed in table 2 have been under observation for approximately 2 years on the grounds of the Agricultural Experiment Station at Santiago de las Vegas, where *Elsinoe* occurs regularly

⁶ JENKINS, A. E. (See footnote 2.)

TABLE 2.—Inoculations on several species of leguminous plants other than lima bean, 1931

Species	Part inoculated	Length of leaflet (cm)	Date inoculated*
<i>Canavalia ensiformis</i>	Tender leaflet	2.5	Mar 27
	Tender leaflet, lower surface	3.5	Apr 2
	Tender leaflet, upper surface	5	Apr 9
	Tender leaflet and end of shoot	1.5	Apr 15
	Tender leaflet, lower surface	2	Do.
	do.	2	Do.
<i>Canavalia gladiata</i>	Tender leaflet, both surfaces	3.5	Aug 12
	Tender leaflet, lower surface	3.5	Aug. 1
	Tender leaflet, both surfaces	2	Aug 7
<i>Dolichos lablab</i>	Tender leaflets	2.2	Aug 30
<i>Phaseolus vulgaris</i>	Tender leaflet	3.7	Oct 4
	Tender leaflet, both surfaces	2	Mar. 25
<i>Phaseolus vulgaris</i>	do.	4	Apr. 9
	Tender leaflet, lower surface	1.5	July 15
<i>Stylobium decringianum</i>	do.	2.5	Do.
	do.	5	Aug. 12
	do.	1.5	Aug. 30

* Results of all inoculations were negative

to some extent on lima beans; but no infection has been found. In addition, *C. ensiformis* has been grown simultaneously for 6 months among lima beans during the present season (November 1931 to April 1932) at El Cano, Habana. Actual counts of spotted pods showed that infection on the lima beans by *Elsinoe* increased from a small amount during the first month or two to from 90 to 100 percent by the first of April. No evidence of the disease could be found on *Canavalia*.

The only other member of the genus *Elsinoe* described on a legume is *E. calopogonii* Syd., reported on *Calopogonium caeruleum* in Brazil.⁷ On August 9 and 10, 1932, inoculations were also made on this legume growing on the station grounds and showing no infection by *Elsinoe*. Six leaflets ranging from 1.5 to 3.5 cm in diameter were inoculated. These remained free from infection, whereas similar inoculations made at the same time on lima beans in pots in the plant-quarantine house became more severely infected than those referred to earlier in this paper.

It thus appears that the species of *Elsinoe* causing lima-bean scab will not infect either *Canavalia ensiformis* or *C. gladiata*, and that *E. canavaliae* is not distributed in Cuba and Puerto Rico on either species of *Canavalia*; moreover, it is evident that the lima-bean organism is not pathogenic on *Calopogonium caeruleum*, on which *E. calopogonii* has been reported. As previously explained, *E. canavaliae* was originally described on *Canavalia gladiata* in the Orient, and has been reported there on *C. ensiformis*; however, the latter host may have been confused with *C. gladiata*.⁸

TAXONOMIC DISCUSSION

In identifying the *Elsinoe* on lima bean it was pointed out that *Elsinoe* had not been recorded on lima beans in the Orient,⁸ although collections of other fungi on this host had been made there. *Elsinoe*

⁷ SYDOW, H., and SYDOW, P. FUNGI AMAZONICI A CL. E. ULE ICTI. Ann. Mycol. 14: 65-97, illus, 1916. JENKINS, A. E. (See footnote 2.)

has since been found on fresh cultivated lima beans from Mexico⁹ collected in 1931 and on dried phanerogamic herbarium specimens of both wild and cultivated lima beans from central Mexico¹⁰ that were collected from 1890 to 1925. Its presence on lima beans on the mainland of the Western Hemisphere suggests that it has long occurred here on this native plant.

Through correspondence with botanists and examinations, particularly of phanerogamic herbarium material, the junior writer has made an additional effort to learn whether a species of *Elsinoe* occurs on lima bean in the Orient or on *Canavalia* in tropical or subtropical regions of the Western Hemisphere. Thus far only negative data have been obtained. As already indicated, the lima-bean organism evidently does not infect *Calopogonium caeruleum*, on which the only other named species of *Elsinoe* occurring on a legume has been described.

The data herein presented certainly indicate that the lima-bean fungus is at least physiologically different from *Elsinoe canavaliae* and *E. calopogonii*. So far as known, all three fungi are similar morphologically though of different known distribution. Pending further investigations, particularly of the two described species, when material is available, it is convenient now to classify the lima-bean organism also as a distinct species. If warranted by data gained from additional studies of these organisms, possibly it will be preferable later to treat them as varieties or physiological forms of *E. canavaliae*, the first species of *Elsinoe* to be described on a legume.

TECHNICAL DESCRIPTION

Elsinoe phaseoli Jenkins, n. sp.

Conidial stage, *Sphaceloma*. Conidiophores aggregated in center of young lesions or developed in marginal zones of older lesions, at first hyaline, then dusky or dark colored, unbranched, blunt or pointed, continuous or 1-septate, closely compacted, forming minute acervuli or sporodochia, or more or less effuse, arising from hyaline or colored hyphae passing through the disrupted epidermis parallel to the surface of the leaf, 20μ or less in length by 4μ in width, bearing conidia at their tips; some conidia minute spherical, hyaline, others ovoid or oblong-elliptical, biguttulate or not, hyaline, becoming dusky, reaching 10μ by 4μ ; ascomata mostly epidermal, becoming erumpent, often composing small punctiform groups, 140μ , more or less, across; asci ovoid or subellipsoid, becoming obclavate with the rupture of the gelatinous outer wall, unruptured asci up to 30μ by 40μ , eight-spored with spores borne parallel to each other in the ascus; ascospores oblong-elliptical or sausage-shaped, basal part sometimes more obtuse than apical, hyaline, but may be stained red or brownish from coloring matter in matrix, becoming 3-septate, or frequently muriform, by formation of a septum in one of the central cells, 13μ to 15μ by 5μ to 6μ , sometimes breaking apart at median septum, germination by means of sprout conidia; both asci and conidia may swell considerably, asci becoming constricted at cross walls and conidia becoming at least 1-septate and constricted at septum.

Status conidiophorus, *Sphaceloma*. Conidiophoris primum hyalinis, dein fuscis vel fuscis, non ramosis, obtusis vel acutis, continuis vel uniseptatis minutas compactos acervulos formantibus, vel effusis, $20\mu \times 4\mu$, conidia apice gerentibus; conidiis aliis minutis, globosis, hyalinis, aliis ovoideis vel oblongo-ellipticis, biguttulatis, fusciscentibus, $10\mu \times 4\mu$; ascomatibus plerumque subepidermicalibus erumpentibus saepe caespites parvos, punctiformes 140μ diam. formantibus; ascis ovoideis vel subellipsoideis $30\mu \times 40\mu$, octosporis; ascosporis parallele dispositis, oblongo-ellipticis vel allantoideis, basim versus obtusis, hyalinis vel ex coloratione matricis brunneolis, 3-septatis, saepe ex septo longitudinali unae cellulae centralis muriformibus, 13μ – $15\mu \times 5\mu$ – 6μ , interdum ad septum rumpentibus; ascis et conidiis multo inflatis, ascis septo constrictis, conidiis ad minimum uniseptatis, septo constrictis.

⁹JENKINS, A. E. ADDITIONAL DATA ON THE RANGE AND PREVALENCE OF LIMA-BEAN SCAB. (Phytopathological Note.) Phytopathology 21: 559. 1931

¹⁰JENKINS, A. E. FURTHER STUDIES OF LIMA-BEAN SCAB. Phytopathology 23: 662-666, illus. 1933.

Distribution.—On wild or naturalized and cultivated lima bean (*Phaseolus lunatus macrocarpus* Benth.), Nayarit and Sinaloa, Mexico; El Salvador, Nicaragua, Costa Rica, and Guatemala, Central America; Cuba, Puerto Rico, Dominican Republic, and possibly Jamaica.

Symptoms.—Disease characterized by hyperplastic lesions on leaves, stems, and pods. Leaf lesions circular to irregular, usually raised on upper surface, sometimes confluent, ranging from mere dots to about 5 mm in diameter, frequently visible on both leaf surfaces; commonly vinaceous buff.¹¹ Stem lesions usually raised, ranging from minute spots to elliptical or elongate areas 1 cm or more in length, where confluent sometimes covering stem for a distance of 2 cm; generally vinaceous buff, sometimes bordered by purplish brown. Pod lesions often swollen, circular to irregular, ranging from mere flecks to lesions 1 cm in diameter; in general, younger lesions brick red to Kaiser brown; somewhat older lesions light cinnamon drab bordered with diamond brown; mature lesions avelaneous, to lighter at center, with reddish to purplish border. Surface of lesions may be black or nearly black owing to growth of *Elsinoe* thereon.

Type specimen.—On cultivated lima bean, Wajay, Havana, November 5, 1929, C. Aguiar. This specimen and others deposited in mycological collections of Bureau of Plant Industry. Culture 254, A. E. Jenkins, isolated from specimen just cited, has been deposited in Centraal Bureau voor Schimmelcultures, Baarn, Netherlands.

SUMMARY

Inoculation tests were made on various legumes in the plant-quarantine house at the Agricultural Experiment Station, Santiago de las Vegas, Cuba, in 1930, with a culture of *Elsinoe* causing scab of lima bean, earlier identified tentatively as *Elsinoe canavaliae* Rac. The infection period was limited to approximately 24 hours. The cotton-plaster method (described) was used and infection readily obtained on leaves and pods of both wild or naturalized and cultivated varieties of lima bean. The cultivated varieties included Fordhook Bush, Henderson Bush, Henderson Pole, and Challenger Pole. Infection occurred on both surfaces of leaflets measuring from 1.5 to 5 cm in length when inoculated, and on tender pods measuring from 1 to 3.25 cm in length when inoculated. In general, the lesions became visible in from 5 to 9 days. Lima-bean plants that were inoculated by being sprayed with water containing bits of a culture became infected in 7 days when covered with bell jars during the infection period, but when not so covered the results were negative.

No infection resulted in inoculations by the cotton-plaster method on *Calopogonium caeruleum*, *Dolichos lablab*, *Phaseolus vulgaris*, *Pisum sativum*, *Canavalia gladiata*, *C. ensiformis*, and *Stizolobium deeringianum*.

The lima-bean *Elsinoe* is now better known than either of the two other species of *Elsinoe* reported on legumes, namely, *E. canavaliae*, occurring on *Canavalia* in the Orient, and *E. calopogonii*, on *Calopogonium* in Brazil. For the present, or until these two described species are studied further, it seems advisable to regard the *Elsinoe* on lima bean as a distinct species. The name *E. phaseoli* Jenkins is therefore proposed for it, and a technical description is given

¹¹ Color readings are based on the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D.C. 1912.

MODE OF ENTRANCE AND PERIODS IN THE LIFE CYCLE OF *CRONARTIUM RIBICOLA* ON *PINUS MONTICOLA*¹

By H. G. LACHMUND²

*Pathologist, Division of Forest Pathology, Bureau of Plant Industry, United States
Department of Agriculture*

INTRODUCTION

The range of the white pine blister rust (*Cronartium ribicola* Fischer) in western North America now extends practically from Alaska to California and from the Pacific coast to Montana. In this greatly enlarged range the rust occurs on new hosts under different climatic and environmental conditions. Investigations of the disease in the West during the last 10 years have resulted in the accumulation of a large volume of field data. The present paper reports the results of studies of the early stages of the rust on native western white pine (*Pinus monticola* Doug.), the chief aecial host in the new range.³ These stages are of special importance, since during incubation the rust is inconspicuous or unrecognizable and the extent and intensity of infection are therefore in doubt. It is primarily in the early stages that the rust has been widely distributed on shipments of seedling white pine.

MODE OF INFECTION

The mode of infection of the pines by *Cronartium ribicola* was not known until it was demonstrated by the experiments of Clinton (1)⁴ and Clinton and McCormick (2, 3) in 1917 and 1918. Inoculations by Klebahn (5) in 1903 and by Tubeuf (16) in 1914 had produced the typical infection spots in the needles, but the connection of these spots with infection in the bark was not understood. Klebahn (5) was not sure how the fungus entered the bark. He stated in 1918 (6) that although it had been shown that the needles could become infected it had not been shown that the mycelium grew from them into the bark. Tubeuf (16) believed that entrance was effected usually by direct infection of the young shoots. McCubbin (10, p. 95), from field observations, concluded that most of the infection occurred in the bases of the leaf fascicles. The work of Clinton and McCormick (3) demonstrated that the germ tubes entered through the stomata of the needles and that the typical mode of infection of the bark was by growth of the hyphae down through the vascular bundles from the infection spots on the needles.

It is still considered possible that the rust may sometimes infect the young bark directly. Spaulding believes that such is the case

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³ The studies were made chiefly in southwestern British Columbia, where the disease has been longest established. Because of the diversity of conditions represented in this territory, it is believed that the results secured there, supplemented by preliminary data from the newer sections, will apply fairly closely to the entire present range.

⁴ Reference is made by number (italic) to Literature Cited, p. 804.

(13, pp. 26-27) but that most infection (14, p. 594) occurs in the manner described by Clinton and McCormick. Although unsuccessful in their attempts to infect the bark directly, Clinton and McCormick (3, p. 441) believe that direct infection may occur to a slight extent in bark that is young enough to have stomata through which the germ tubes of the sporidia may enter.

Field evidence in the present studies supports the conclusions of Clinton and McCormick (3) and indicates that the mode of infection in *Pinus monticola*, as in other aecial hosts of *Cronartium ribicola*, is through the needles. After favorable infection years, the golden yellow to brownish spots that mark the location where infection took place in the needle are generally abundant wherever much infection of pines is in progress. The outstanding field evidence observed by the writer showing that the bark is typically infected through the needles in *P. monticola*, however, is as follows: The first characteristic yellow to orange discoloration or swelling in the bark, which marks the appearance of the incipient canker, almost invariably develops symmetrically around a single needle bundle. Aside from its central location in the discolored area this bundle is generally distinguished by a marked swelling of its base. An examination of the needles of this bundle will usually disclose at least one definite needle infection spot. Other needle bundles may occur on the discolored area, but, except where there is evidence that the rust has entered from more than one needle bundle, their bases are generally not swollen. Frequently the central bundle, through which infection entered, persists considerably longer than the rest. Where all needles have fallen the enlarged scar of this bundle is generally conspicuous, or at least is recognizable, in the center of the discoloration.

Indirect evidence that the mode of infection is through the needles is found in the distribution of the incipient cankers resulting from any given year's infection on the various years' internodes exposed to the infection (7, 8). If the internodes formed the year of infection are designated as *a*, those of the preceding year as *b*, those 2 years older as *c*, etc., the occurrence of needles at the normal time of infection in late summer and early fall is limited primarily to *a*, *b*, *c*, and *d*, although *e* will have some, *f* may have a few, and *g* or even *h* may rarely have a few. The occurrence of the incipient cankers is confined to internodes within these age limits, i.e., to growths upon which needles may occur at the time of infection, and an average of over 99 percent of the cankers develops on *a* to *d* where the chances are best for entrance through the needles. Based on tabulations of approximately 6,000 cankers from study areas throughout the rust's western range, the average distribution of the incipient cankers on the pines is as follows: *a*, 10— percent; *b*, 53+ percent; *c*, 31+ percent; *d*, 5+ percent; *e*, 0.5 percent; *f*, trace; and *g* and older internodes, 0 percent.

The sparsity of the cankers on *e* and older internodes corresponds with the sparsity of needles on these internodes. Most of the needles on *d* are cast at the end of the infection season, so that relatively little time is available for the entrance of infection through these needles. As might be expected, therefore, if infection enters through the needles, *d* has only a relatively small proportion of the cankers. *c* loses part of its needles at the end of the infection season and most of the remainder 1 year later. The chances for the entrance of in-

fection through the needles on *c* are therefore relatively good. The proportion of the cankers on this growth averages 31 percent. *b* holds its needles a full year longer than *c* and has 53 percent of the cankers. The needles on *a* are held a full year longer than those on *b*; in other words, all of them are normally held 3 full growing seasons, including the season of infection, and the majority are held 4 seasons. The cause of the small proportion of cankers on *a* appears to be that its needles, i.e., the current season's needles, are resistant to infection (8).

Those who have stated or suggested that infection enters the bark directly rather than through the needles, or that direct infection of the bark occurs at all, have generally agreed that it will occur primarily on the new shoots where stomata are present and where the tender young bark permits the entrance of the germ tubes of the delicate sporidia. On this basis *a* should have most of the cankers; actually it has only about 10 percent as compared with 53 percent on *b*, 31 percent on *c*, and 5 percent on *d*. The only explanation for this is that the mode of infection is through the needles.

INCUBATION PERIOD

DEFINITION

The term "incubation period" in its ordinary application refers to the time between infection and the appearance of disease. Since *Cronartium ribicola* infects through the needles and the yellowish or brownish spots on the needles represent the first symptoms of the disease, the incubation period might strictly be defined as the time from infection to the appearance of these spots. But there is considerable variation in the development and character of the spots. In examining *Pinus monticola* under natural conditions it is generally possible to be reasonably certain of the identity of the spots after they have become enlarged and have assumed the characteristic golden-yellow color. However, before this stage is reached and afterward when the infected needle tissues begin to die and turn brown, or where the development is atypical, there is frequently no definite macroscopic means of distinguishing the blister rust from spots resulting from other causes. Consequently, even when no other spots are present, there is always a considerable proportion of the blister-rust spots that cannot be identified with certainty. Furthermore, where, as must often be the case, the fungus fails to reach the bark before the needle is shed, the infection in the needle is of no practical significance. In exceptional cases, moreover, it is possible that infection takes place directly in the young bark (3, p. 441; 13, pp. 26-27). For these reasons, the time required for the development of the needle spot is an unreliable and unsatisfactory measure of the incubation period of blister rust, at least on native *P. monticola*.

The next stage in the development of the fungus is characterized by discoloration and swelling of the bark. After varying periods these symptoms are followed by the production of pycnia and later of aecia.

In conformity with the most generally accepted practice in the treatment of the early stages of the aecial forms of *Cronartium*, Spaulding and earlier writers (13, pp. 24-26) have considered the incubation period of *C. ribicola* on its aecial hosts as the period from infection to the production of aecia. Such a definition is of course necessary and agrees exactly with the meaning of the term when, as

is frequently the case with other species of the genus, the disease cannot be recognized earlier. The symptoms of infection by *C. ribicola*, however, are usually apparent on most of its pine hosts considerably before this time. In *Pinus monticola* the conspicuous yellow-to-orange discoloration in the normally smooth green bark readily establishes the identity of the incipient canker at a very early stage and generally before the formation of pycnia. Since the time elapsing between the appearance of the incipient canker and the production of aecia is highly variable, to define the incubation period as extending from infection to the production of aecia would not only make the period unduly long, variable, and indefinite, but would complicate the subject unnecessarily. The incubation period for *P. monticola* is therefore defined as the period from infection to the appearance of discoloration or swelling of the bark sufficient for a ready and accurate macroscopic diagnosis.

LENGTH OF THE PERIOD IN TREES BEYOND THE SEEDLING STAGES

In the natural infection areas of the western range of the rust, the trees vary in size from seedlings to veterans. Generally the best areas for study have been located in young reproduction ranging up to 30 or 40 feet in height, although trees of all sizes have been included. On the youngest seedlings, as will later be shown, the incubation period of the rust is shorter than on trees more than about 3 feet high or 8 years of age. Since most of the foliage is on the larger trees, however, it is obvious that most of the infection will be found on trees beyond the height and age limits just specified. On trees beyond these limits the writer has never seen an incipient canker on internodes younger than those formed two seasons before the season of examination. Minute orange discolorations have occasionally been observed about the bases of needle bundles in bark formed the year preceding that of examination, but it was impossible to determine from macroscopical examination whether they were young infections or whether they were caused by aphids, which often induce a similar type of discoloration. Since the symptoms were uncertain and the discolorations could not be definitely classified, these cases were excluded from the calculations of the length of the incubation period on the older trees.

Throughout the present range of the rust on *Pinus monticola* the first teliospores usually begin to form on *Ribes* during June. Their production reaches maximum proportions in late July, August, or September, depending on the weather. By the end of November *Ribes* plants are generally defoliated and the teliospores have germinated. Late rains saturate the surface duff and assure germination of any teliospores that may remain on the fallen leaves. Consequently the amount of infection that results from fallen leaves some time after general defoliation, that is, after about December 1, is negligible, and for practical purposes may be disregarded. Infection of pines, therefore, may occur from about June to November, inclusive, and is generally heaviest in August, September, or the first part of October, after most of the teliospores have formed.

The earliest formation of incipient cankers recorded in general (i.e., on trees more than about 3 feet in height or 8 years of age) has been in April of the second year following that of infection. Thus the minimum incubation period in general for *Pinus monticola* is at least 16 months, or from infection at the end of November of one year

to April of the second succeeding year (fig. 1, *A*). Since infection usually occurs several months before the end of November, the incubation period is probably nearer 18 months.⁵

Observations of waves of canker formation resulting from single years of infection at various localities that represent a wide diversity of conditions in southwestern British Columbia and also include areas from the newer sections have shown that most of the incipient cankers appeared between May and November of the second year after the year of infection. Since most of the infection occurs between late

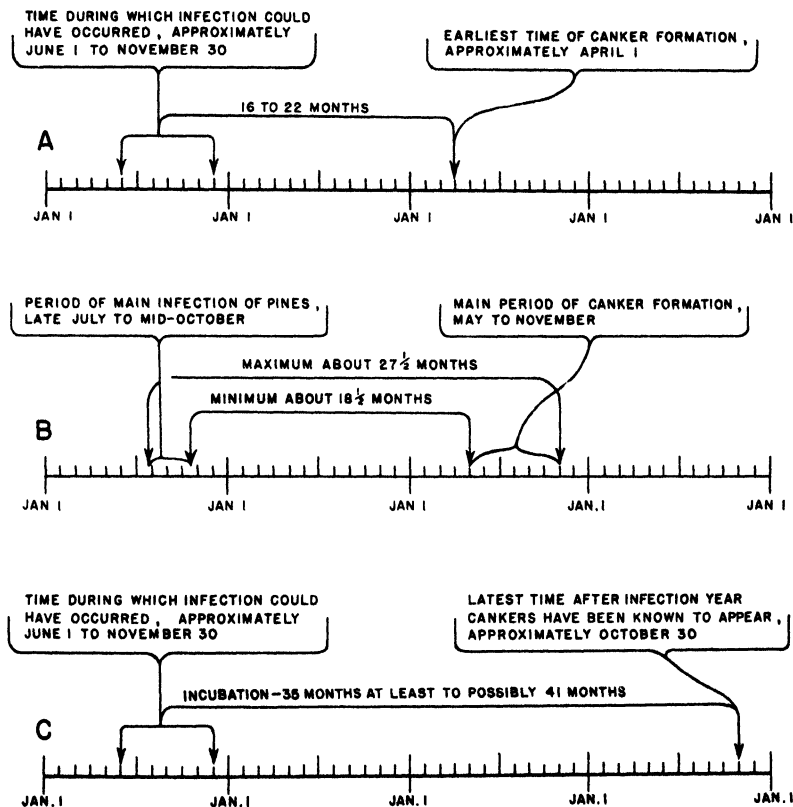


FIGURE 1. Incubation period of *Cronartium ribicola* on *Pinus monticola*: *A*, Minimum incubation period (see footnote 5); *B*, maximum range of usual incubation period; *C*, maximum incubation period.

July and mid-October, the usual incubation period is seen to range at most from 18½ to 27½ months (fig. 1, *B*).

Specific studies of individual infection waves have shown that for most of the cankers the incubation period averaged from about 21 to 24 months. At Revelstoke, British Columbia, in 1922, for example, sporidia production and infection were concentrated in September, and the resultant canker formation occurred in June, July, and August of 1924, a period of about 20 to 23 months.

⁵ E. L. Joy, of the Division of Blister Rust Control, reports finding recently on the larger trees in infected areas in Idaho definite incipient cankers as early as September 15 of the year following the infection year. This would reduce the minimum incubation period for most trees to between 9½ and 15½ months, probably to about 12 to 13 months.

On an experimental plot at Apex, British Columbia, the infection was transmitted from two well-isolated and artificially infected bushes of *Ribes viscosissimum* Pursh in 1928. Detailed records of the development of infection on the *Ribes* bushes and of weather conditions showed that the main sporidia production and most favorable conditions for infection of the pines occurred in September. Between May 1930 and about August 1931, 203 incipient cankers developed. Most of these were produced in July, August, and September 1930, the incubation period being between about 21 and 24 months for the majority of the cankers.

At Thurston Bay, British Columbia, in 1922, observations of *Ribes* infection and of weather conditions showed that the main infection of pines must have occurred in October. The resultant canker formation was concentrated in September and October 1924 after a lapse of about 22 to 24 months. On a similar basis, the incubation period was calculated at between 21 and 22 months after infection at Thurston Bay in 1924; between 20 and 22 months after infection at Qualicum Beach, British Columbia, in 1924; between 21 and 23 months after infection at Qualicum Beach in 1927; between 22 and 26 months after infection at Daisy Lake, British Columbia, in 1923; and between 23 and 25 months after infection at Owl Creek, British Columbia, in 1924.

Frequently a considerable proportion of the cankers resulting from a given year's infection make their appearance in the third growing season after that of the infection year. For example, about 10 percent of the incipient cankers resulting from the 1921 infection at both Chee Kye and Daisy Lake, British Columbia, did not form until April 1924. At Revelstoke about 25 percent of the cankers resulting from the 1922 wave of infection did not appear until the growing season of 1925 and some of them were still forming in August of that year. The incubation period for these cankers was 33 months at the minimum and 35 months if counted from September 1922, when conditions were most favorable for infection. Similarly, at another infection area several miles north of Revelstoke, at least 20 percent of the cankers of 1927 origin did not appear until the growing season of 1930, some of them forming as late as October. The incubation period for these cankers was not less than 35 months and was probably closer to 38 months. Assuming that some of the infection occurred in June 1927 and that some of the cankers appearing in October were the result of this infection, the incubation period for such cankers would be 41 months (fig. 1, C).

What the maximum incubation period may be in individual cases is not known. Incipient cankers are occasionally found forming on internodes as old as 9 years, which would make a possible maximum of 9 years in incubation. However, since infection occurs through the needles and *Pinus monticola* holds some of its needles occasionally as long as 8 seasons, it is far more likely that such cankers have entered these internodes through persistent needles long after the year in which the internodes originated. It is improbable that the incubation period is ever more than 4 years. As already shown, the incubation period for most of the cankers is between 20 and 26 months and ranges for decreasing numbers of cankers to about 38 or 39 months, with a possible maximum of about 41 months.

It should be borne in mind that in nature the growth of the fungus is very much retarded during the colder season. Measurements of cankers (not reported here) indicate that growth during the months intervening between growing seasons—or, roughly, from October to March, inclusive—is but from a fifth to a sixth of the growth made between April and September, inclusive. In other words, growth is practically as great during a single month of the growing season as during the entire 6 months of late fall, winter, and early spring. Variation in the month of infection, therefore, will act disproportionately upon the length of the incubation period, generally tending to shorten the period if infection occurs early and lengthen it if infection occurs late.

Thus, if infection occurs at the end of June, the individual infection will have the following months to develop to the point of canker incipency: the 3 active months of July, August, and September; the following 6 inactive months, equivalent to 1 active month; 6 active months; 6 more inactive months; and 6 more active months—a total of 27 months, active and inactive, or the equivalent of 17 active months, from the time of infection to the end of the second season following that of infection. If infection occurs at the end of July, the equivalent of 17 active months would extend the time to the beginning of the third growing season following that of infection, making a total of 32 months, active and inactive. However, if only 16 active months are required for incubation, infection at the end of July would permit the formation of the incipient canker at the end of the second season following that of infection after a total of only 26 months.

Recapitulating the former conclusions as to the general length of the period required for incubation by calculating the 6 months from October to March as the equivalent of 1 active month and each month from April to September as 1 active month, the period would range from a possible minimum of about 8⁶ to a possible maximum of 25 active months, and average between about 10 and 16 active months.

Undoubtedly it is not strictly accurate to give equal weight to each of the active months from April to September. Probably in May, June, and July, or in June, July, and August, growth activity of the rust is somewhat greater than in the other months of this period. This point has yet to be fully studied. Undoubtedly such differences will vary with the weather and locality and will increase with altitude and northward latitude. Obviously these differences will accentuate the disproportionate effect of variation in time of infection on length of incubation period.

At the upper elevations for *Pinus monticola*, where the growing season may be as much as 2 months shorter than in the main range of the species, the active season for the rust should be calculated at 5, or even 4, instead of 6 months, depending on local conditions, and the remainder of the year as the equivalent of 1 active month. It is to be expected that reduction of the active season for the rust will be reflected in a longer period for incubation. This tendency has been very noticeable in the study areas of the highest elevation and shortest growing seasons from which data are available; in these areas the appearance of considerable proportions of the cankers in the third

⁶ In the case of the incipient cankers reported by E. L. Joy (see footnote, p. —), the lowest possible minimum would be about 6½ active months, or from Nov. 30 of one year to Sept. 15 of the next.

season after infection is a frequent occurrence, whereas in the study areas, at about sea level, where the growing season has been longest, it is a rare occurrence.

In this connection it should be stated that all these higher areas are situated much below the upper altitudinal limits for *Pinus monticola*. The data on incubation at or about these limits is too fragmentary to form a basis for conclusions. It is possible that at these extremes a large majority of the cankers may not appear until the third season after infection.

Of the areas studied those of relatively higher elevations and shorter growing seasons more closely resemble the commercial range of *Pinus monticola* in general climatic conditions than do the sea-level areas. It is interesting to note, therefore, that preliminary data from the commercial range in Idaho also indicate a frequent and considerable extension of canker formation into the third season after infection.

Another factor which might influence the incubation period is the difference in growth rate of the fungus induced by variations in the thickness and thrift of the bark. The growth of the canker is strongly favored by the thickness, and, to a large degree, by the vigor of the infected bark, and consequently is more rapid in the larger, thriftier branches and stems than in the smaller, slower-growing twigs and branches. It might, therefore, be expected that the incubation period would be shorter and that the incipient cankers would appear earlier on the larger and thriftier branches. H. N. Putnam, of the Division of Blister Rust Control, informs the writer that he has observed evidence of such a relationship in a number of areas. The writer has not been able to establish definite evidence of it in his own work, but he has observed a noticeable difference in the size of the initial discolorations, which in general depends on the size and thrift of the part affected.

LENGTH OF THE PERIOD IN SEEDLINGS

On very young trees, ranging up to 4 or 5 years of age, incubation generally requires a full year less than on the older trees. This was first observed by the writer in 1924 in an experimental planting of five hundred 3-year-old nursery stock of *Pinus monticola* that had been made at Daisy Lake, British Columbia, in 1923, by the Dominion Department of Agriculture.⁷ Since then the same phenomenon has been noted as typical of the development of the rust on trees of this age class.

The trees of the experimental planting just mentioned were healthy transplants from the United States Forest Service nursery at Wind River, Wash. No instance of white pine blister rust was then known at the nursery or in the vicinity of it; it is certain that the trees were not exposed to infection there, for western white pines remaining at the nursery did not develop infection.

The plot in which the trees were set out at Daisy Lake was in the center of an infection area close to heavily infected *Ribes*. Planting was done on April 10, 1923. Between mid-June and defoliation of *Ribes* in the fall, the trees were subjected to heavy infection. Data on weather and on the production of sporidia indicated that the main infection of the pines occurred in August.

⁷DAVIDSON, A. T. RESULTS OF PAST SEASON'S WORK, PRESENT SITUATION, AND FUTURE PLANS. Report of Proceedings, Meeting of Trustees, Western White Pine Blister Rust Conference, held in Spokane, Wash., Jan. 7 and 8, 1924, pp. 7-18. [Mimeographed.]

On June 18, 1924, incipient cankers resulting from this infection had begun to appear. From that time until the end of the growing season, 265 of these cankers had formed. It was evident from the rate at which the cankers grew that the first of them must have appeared about 2 weeks before the first examination of the trees on June 18. The incubation period for these first-formed cankers was not more than 11½ months, i.e., from mid-June in 1923, when the first teliospores were produced, to the 1st of June 1924, when the cankers made their appearance, or the equivalent of less than 7 active months. Assuming that the infection occurred in November, the incubation period may have been as short as 6 months, the equivalent of only 3 active months. This must represent close to the minimum limit for the incubation period of the rust under any conditions. In the present case, since the main infection occurred in August, the minimum period of incubation was probably nearer 9 months, or the equivalent of 4 active months.

Most of the cankers appeared in August or September. Since the majority of these unquestionably resulted from the main infection in August of the preceding year, the incubation period for the majority of the cankers ranged from 12 to 13 months, or from 7 to 8 active months.

For the cankers which made their appearance at the end of the season, the possible range of the incubation period was from 11 to 17 months. Estimated from the time of the main infection, the incubation period was about 14 months, or the equivalent of about 8 active months.

The degree to which the incubation period of the infections of 1923 extended beyond these limits could not be exactly ascertained. When the pines were examined again in November 1925 it was found that many new cankers had appeared that year, almost equaling in number those formed in 1924. But it was evident from the proportion of the cankers found on the 1924 internodes that much additional infection had occurred in 1924, and it was impossible to determine exactly what proportion of these new cankers were of 1923 origin and what proportion were of 1924 origin. Apparently, judging from their relative distribution on internodes of different years and from their stage of development, about one third to one half of them were of 1923 origin. Those falling in this latter category were generally more advanced in development than the rest and had evidently appeared some time earlier in the season.

There was no evidence to indicate that any of the more recently formed cankers were of 1923 origin. It is probable, however, that a few of them were, and that incipient canker formation on these youngest classes of trees may extend to well toward the end of the second season after infection, just as in the older classes of trees it sometimes extends to the end of the third season after infection. This would bring the possible range in the period on the young classes of trees up to a possible maximum of about 30 months. There is no evidence, however, to show that the period extends to any appreciable degree into the third season following infection, and incubation may be considered in general to require at least a year less than on the older trees.

TIME REQUIRED FOR PRODUCTION OF PYCNIA

Observations during the entire period of study indicate that the formation of pycnia on *Pinus monticola* is strongly influenced by temperature. Depending on local and seasonal climatic conditions, it may begin as early as May and continue as late as November. In warmer localities near sea level it usually begins in June, is heaviest in July and August, and subsides when the weather turns cooler, usually in September; but periods of warm or hot weather may induce a considerable resumption of activity in the production of this spore form in October or even November. At higher elevations the season of production generally begins later by several weeks or more and ends earlier by a corresponding period.

If the incipient canker forms before the middle of the period during which pycnia normally appear, pycnia are almost invariably produced upon it the same year. If it forms during the period of maximum pycnial activity, it may bear pycnia almost immediately. If it forms later in the season, pycnia are generally not produced upon it until the following summer. The period required for production of pycnia after the formation of the incipient canker, therefore, may vary from less than a month to as high as 10 months, depending on seasonal conditions and the time the canker forms. Consequently the period from infection to the production of pycnia is, roughly, from 1 to 10 months longer than the incubation period. The equivalent increase in active months would be 1 to 5 of such months, the maximum being calculated as from the first of September to the first of July of the following year.

TIME REQUIRED FOR PRODUCTION OF AECIOSPORES

In the western range of the rust, aeciospore production on *Pinus monticola* varies widely with seasonal conditions and locality. At low elevations it may begin as early as the last of February, but usually it does not begin until the latter part of March or the first part of April. At high elevations near the upper limits of occurrence of the pine, it may not begin until as late as July, but apparently it is usually under way by the first of June. As stated earlier, however, relatively few data are available from these marginal regions. The heaviest production through the main range of *Pinus monticola* in British Columbia occurs between early April and mid-June. Fairly heavy production has been observed in a few cases, however, to extend well into July or even into August. At high elevations the main production appears to occur usually between mid-May and mid-July and to be concentrated within the relatively short period of a month or less. At lower elevations, also, the period of heaviest production is often short.

Rather frequently at the lower elevations a sporadic and light resumption of aeciospore production may occur in late fall. This has been noted at a considerable number of infection localities at such elevations in several different years in October or November. It usually occurs under the influence of a period of warm and open fall weather in years having an early spring. It has not been noted at high elevations. Practically, it is of little importance, since the aecia are small and dwarfed and occur on only a fraction of 1 percent of the cankers at a time when *Ribes* defoliation is generally in progress

and the spores can cause little or no infection. Nevertheless, it prolongs the period in which aecia may be borne to practically the entire growing season and suggests the possibility of the occurrence of incipient canker formation, pycnial sporulation, and aeciospore production all in the same year. No instance of this sort has been actually recorded, however.

On the majority of the cankers aecia are usually produced in the spring of the year following that in which the cankers first bore pycnia. Frequently, however, a large proportion, sometimes a majority, of the cankers do not produce aecia until the second year after that in which pycnia are produced. Observations and tabulations of cankers of various infection waves have shown percentages bearing or failing to bear aecia in the year following that in which pycnia were first produced approximately as given in table 1.

TABLE 1.—*Aecial production in Cronartium ribicola cankers in various western study areas*

[Cankers had borne first pycnia in year preceding that of observation]

Area of infection wave	Year of observation	Cankers observed		
		Total	Bearing aecia	Not bearing aecia
		Number	Percent	Percent
British Columbia				
Thurston Bay	1923	77	51	49
Do	1924	281	55	45
Pender Harbor	1921	134	24	76
Chee Kye	1921	377	89	11
Daisy Lake	1921	143	68	32
Mile 72, Pacific Great Eastern Railway	1924	476	79	21
Revelstoke	1925	184	78	22
Qualicum Beach	1926	65	14	68
Owl Creek	1926	228	36	64
Heaton	1926	171	83	17
Do	1929	65	51	49
Arrow Park	1930	129	64	36
Columbia River plot	1930	1,653	51	49
Eagle Creek plot, Oregon ^a	1930	177	52	48
Average			57	43

^a Data collected by the Spokane branch of the Division of Blister Rust Control, Bureau of Plant Industry, U. S. Department of Agriculture.

Cankers which fail to produce aecia in the year following that of pycnial production usually produce pycnia again the second summer and aecia the next spring. Sometimes, however, some of the cankers do not produce aecia until the third or even the fourth year after that in which they first produced pycnia. Frequently this is caused by the presence on the cankers of secondary parasites which kill the bark on which pycnia were produced before aecia could be formed. However, the same thing has been observed in cankers that were not visibly affected in any way by secondary parasites.

The cause of this hold over in the development of aecia where the bark remains alive is unknown. Craigie (4), working with other heteroecious rusts, has shown that the pycnial stage constitutes an essential sexual step in the life cycle of these rusts. Association of the positive and negative pycniospores in the pycnia is a necessary preliminary to the formation of aecia. The two types of pycniospores are produced in separate pycnia which are usually intermingled on

the same host substratum so that the pycnial fluid containing the spores is easily carried from one pycnium to another by insects, rain, gravity, or other agencies. Any factors that prevent this process generally inhibit the formation of aecia.

Though not yet demonstrated experimentally, the function of the pycnia in *Cronartium ribicola* is doubtless the same as that in the other heteroecious rusts.⁸ If this is true, weather and other physical factors preventing a satisfactory production or distribution of the pycnial fluids and spores will probably be found to be chiefly responsible for the delays in aecial production on cankers failing to produce aecia in the season following that of the formation of pycnia. Parasites upon the pycnia, however, may also play an important part.

In this latter connection it is worthy of mention that *Tuberculina marina* Rostr., a common parasite on the aecia in Europe (9, 15), is also widely distributed and evidently indigenous on the rust in the Pacific Northwest. In certain wet springs this fungus has been abundant at several infection areas here on the aecia, aeciospores, and also on the spots where pycnia had formed the preceding year. It has also been noted abundantly on the pycnia during summer. Although where infection of aecia is heavy aeciospore production and dissemination are considerably reduced, the fungus may actually be of more importance in the reduction of aecia through parasitism of the pycnia. In cases where parasitism of pycnia is extensive it undoubtedly is an important factor in restricting the production of aecia the following spring. In general, however, the occurrence of the fungus in the Pacific Northwest is sporadic.⁹

Cankers affected by secondary organisms may not produce aecia at all. In such cases, however, the canker generally dies within a few years of its formation. Occasionally, the outer portions may die while the rust spreads on down the branch from the lower portions and the secondary organism follows but does not completely overcome the infection. Ultimately, the rust may reach the bole without yet having produced aecia. Here it may produce aeciospores, perhaps 10 or 15 years after pycnia were first produced on the branch. If such an infection enters where the bole is large and the bark is thick and rough, the production of aeciospores may be still further delayed, for such bark greatly retards aecial sporulation and may inhibit it altogether. Infection may also occasionally enter a large trunk directly through a short shoot, killing the shoot before aecia can be produced. In such cases the fungus may vegetate in the thick bark for a period of 5 or 10 years and not produce aeciospores until it has spread from the bole into the thinner bark of some branch.

The period required for the development of aeciospores after the first production of pycniospores is therefore seen to range from a possible minimum of less than 4 months (November to March) up to a possible maximum of perhaps 2 decades or more if secondary-parasite infection is considered, or to over a decade if only the infection through short shoots is considered. In general, it ranges from about 6 months to 2½ years. These periods added to the period required for the production of pycnia make the period from infection to the

⁸ A preliminary report of experimental results with *C. ribicola* on *Pinus monticola* by Pierson (12) indicates that such is the case. Pycnia fertilized by interchange of pycniospores gave apparent fusions of pycniospores with the filamentous hyphae in 11 cases, whereas unfertilized pycnia gave none.

⁹ These relationships have been recently brought out by Mielke (11) in a report of this parasite's western distribution and behavior.

production of aecia an indeterminate one of about 2 to 7 years for trees more than about 3 feet high and 8 years old. In special cases the minimum period on such trees may be a few months less and the maximum 10 to 20 years longer.

A somewhat different method must be used to calculate the usual period from infection to the production of aecia. As stated earlier, a large majority of the incipient cankers make their appearance in the second year after the infection year, and the remainder form in the third year after the infection year. Extensive tabulations indicate that the average incidence of cankers is about 85 percent in the second year and 15 percent in the third year after the year of infection.

Of the cankers that appear in the second year after infection, the percentage producing pycnia varies according to the time of year at which the cankers are formed. The tabulations indicate that the average is about 70 percent. Of the cankers that appear in the third year after infection, an average of about 90 percent produce pycnia. Practically 100 percent of the cankers that form too late in the season to bear pycnia that year, produce pycnia the next year. Of the cankers that produce pycnia for the first time in a given year, an average of about 60 percent produce aecia the following spring, and at least 90 percent of the remainder produce aecia the next spring following.

As calculated from these percentages, the cankers resulting from a given infection year produce aecia for the first time after infection in about the following proportions: 36 percent in the third spring after the year of infection, 43 percent in the fourth spring, 18 percent in the fifth spring, and the remaining 3 percent mainly in the sixth and to some extent in the seventh spring.

As stated earlier, most of the production of sporidia and infection of pines occurs between late July and mid-October. The production of aeciospores begins usually between April 1 and mid-May. Therefore, for the 36 percent of the cankers which first produce aecia in the third spring after the year of infection, the usual period from infection to aeciospore production is approximately $2\frac{1}{2}$ to 3 years; for the 43 percent which first produce aecia in the fourth spring, the period required for aeciospore production is approximately $3\frac{1}{2}$ to 4 years; for the 18 percent which first produce aecia in the fifth season, the period required is between $4\frac{1}{2}$ and 5 years; and for most of the remaining small percentage, the period is between $5\frac{1}{2}$ and 6 years.

On trees less than 5 years old, the minimum period from infection to production of aecia is about a year less than on the older trees, but the possible range of the period will be very much less because of the small size of the parts and the thinness of the bark of the young trees. The maximum period on such stock is about 5 years. The usual periods are $1\frac{1}{2}$ to 2 years; $2\frac{1}{2}$ to 3 years; and, to a lesser degree, $3\frac{1}{2}$ to 4 years for cankers producing their first aecia in the second, third, and fourth spring, respectively, after infection.

SUMMARY

Field evidence indicates that in *Pinus monticola*, as in other white pine species, *Cronartium ribicola* gains entrance to the bark through the needles. Typical needle-infection spots are developed. The initial discoloration, which marks the incipient canker in the bark, is

almost invariably centralized symmetrically about a single needle bundle the base of which is usually swollen and the needles of which have at least one characteristic rust-infection spot. Except for the internodes of the infection season, upon which the needles appear to be resistant, most of the cankers form upon the younger internodes where the needles are most numerous and are retained longest.

The incubation period is defined as the period from infection to the appearance of discoloration or swelling in the bark sufficient for an accurate macroscopic diagnosis. On trees more than 3 feet high and 8 years old, the period for most of the infection ranges from about 20 to 26 months, or the equivalent of 10 to 16 active months, if each month of the growing season, from April to September, inclusive, is considered as 1 active month and the intervening inactive season, from October to March, inclusive, is considered as the equivalent of 1 active month. After infection on such trees, the minimum period required for cankers to form is generally not less than 16 months, or the equivalent of about 8 active months. Recent data collected in Idaho by the Division of Blister Rust Control indicate that in certain cases the extreme minimum might have been as little as 9½ months, equivalent to 6½ active months. The maximum period for the latest-formed cankers observed could not have been less than 35 months and might have been as much as 41 months, the equivalent of 21 to 25 active months. It is not probable that the incubation period ever extends beyond 4 years. It varies considerably, depending on the time of infection and seasonal conditions, and is longer at higher elevations, where the season is shorter, than at lower elevations, where growth activity is greater and extends over a longer period.

On the youngest trees, up to 4 or 5 years of age, the incubation period is about a year shorter than on the older trees. Incipient cankers have been observed to form upon them after a possible minimum of 6 months, or an equivalent of only 3 active months.

Following incubation, the period required for the production of pycniospores ranges from less than a month to 10 months.

Following the production of pycniospores, the period required for the production of aeciospores is usually from 6 months to 2½ years. It sometimes extends over 3 or even 4 years, and on the largest trees under certain circumstances occasionally ranges as high as 10 to 20 years. On the youngest trees it rarely extends beyond 2½ years.

On trees more than 3 feet high and 8 years old, therefore, the total time from infection to production of aecia ranges in general from about 2 to 7 years and may occasionally exceed 20 years. The usual period is between 2½ and 5 years. On trees less than 5 years of age the total period ranges from about 1 to 5 years, and is usually from 1½ to 4 years.

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INCREASE IN GROWTH OF LOBLOLLY PINES LEFT AFTER PARTIAL CUTTING¹

By A. L. MacKINNEY²

Assistant Silviculturist, Appalachian Forest Experiment Station, Forest Service,
United States Department of Agriculture

INTRODUCTION

Information on the growth of trees left after partial cutting, and on the factors affecting it, is of value in planning the management of forest stands. Such information makes it possible not only to predict the growth of trees that have been left by partial cutting but also to select as trees to be left in partial cutting those more capable of benefiting by release. Together with information on other silvicultural characteristics of a species and on economic factors, it forms the basis for applied silviculture. This paper deals with the results of a study of increase in growth at breast height³ of loblolly pine (*Pinus taeda*) trees left after partial cutting.

REVIEW OF LITERATURE

Many workers have presented data on the increase in growth rate of forest trees after release. Marshall (11),⁴ studying the growth of hemlock (*Tsuga canadensis*), found that on an average the trees grew five and one half times as fast in diameter at stump height after release as before. Lutz (9) and Merrill and Hawley (12) found that released hemlock trees showed an increase of from 250 to 350 percent in rate of diameter growth at breast height.

Ponderosa pines (*Pinus ponderosa*) studied by Krauch (8) showed marked increase in diameter growth after they were released by removal of part of the stand. In this instance the percentage increase in diameter growth was found to be inversely proportional to diameter at time of release. Dunning (5), also working with ponderosa pine, found trees with large crowns more responsive to release than small-crowned trees.

Bentley and Recknagel (3) pointed out that red spruce (*Picea rubra*) increased in rate of growth following release. The same tendency was noted for balsam fir (*Abies balsamea*) by McCarthy (10), who found that the small trees showed greater increase in diameter growth than the large ones.

Longleaf pines (*Pinus palustris*) studied by Barrett (1) showed a marked increase in growth after release. Trees that were small at the time of release showed greater increase in rate of volume growth than the larger trees.

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² The field work of this investigation was initiated by C. F. Korstian, formerly of the Forest Service, to whom the writer is indebted for later assistance. Acknowledgment is also due F. X. Schumacher and D. Bruce, of the Forest Service, for valuable suggestions in the statistical analysis, and Mary L. Denoyer, of the Forest Service, for assistance in the computational work.

³ All diameter and basal-area data mentioned in this paper represent measurements taken at breast height (4.5 feet above ground) outside bark, unless otherwise specified.

⁴ Reference is made by number (italic) to Literature Cited, p. 821.

Chapman (4), investigating the growth of several loblolly pines after partial cutting, found a marked increase in rate of diameter growth. He concluded that the rate of increase depended upon the relative length of the crown at the time of release.

CHARACTER OF DATA

The data used in this study were taken during 1927 and 1928 in nine loblolly pine stands on the Atlantic coastal plain of Virginia and



FIGURE 1 --Loblolly pine stand 11 years after it was cut to an elastic 10-inch diameter limit.

North Carolina. The stands had all been partially cut at least 10 years before and had since remained unburned. Figure 1 shows a typical stand. In general they were of the same age (45 to 60 years at the time

of cutting), had the same site index ⁵ (85 feet) according to determinations in near-by stands, had alike been cut to an elastic 10-inch diameter limit, and were approximately the same in density of stocking by basal area (50 to 70 percent of normal values (13) at time of cutting).

The diameter distributions in the several stands at the time of cutting were estimated from tree and stump tallies, on the basis of increment borings. The stand tables so reconstructed undoubtedly include errors due to the rough method of taking stand and stump tallies and to the lack of data on mortality of trees since the time of cutting. In general, however, they indicate a high degree of similarity

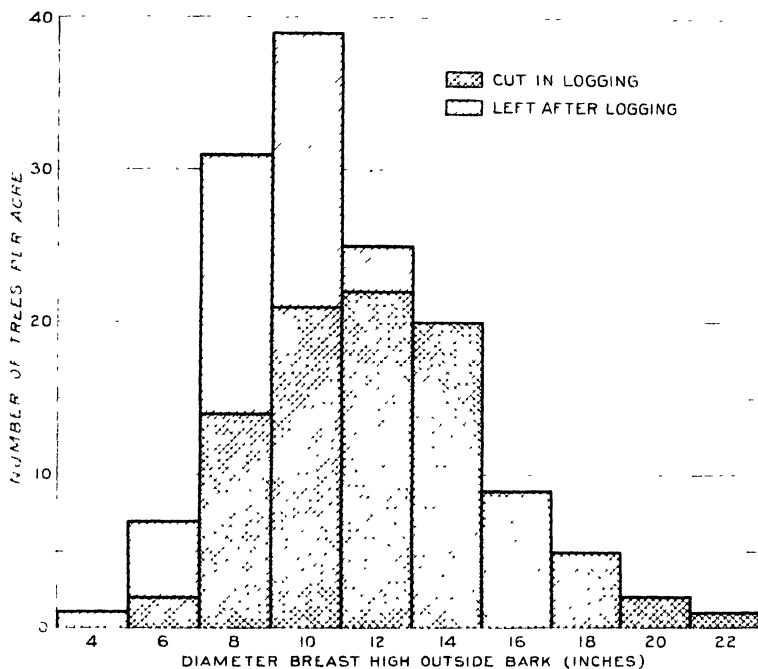


FIGURE 2 - Average distribution of trees cut and trees left in logging on study areas, by 2-inch diameter classes, according to reconstructed stand tallies

among the nine stands as to density of stocking and as to representation of various diameter classes. Average stand conditions on the nine areas are presented graphically in figure 2.

A selected portion of each of the nine stands was examined in detail, the following data being taken for each of 1,464 trees:

Measurement	Method
Diameter at breast height.....	Diameter tape.
Total height.....	Abney level.
Height to base of crown.....	Do.
Crown width.....	Estimated to nearest foot.
Growth in stem radius at breast height for 5 years before and 10 years after release.	Measured from increment cores taken on south side of trees.
Total age.....	Increment cores.
Number and size of trees cut and trees left within 30 feet of the tree studied, and their position in relation to it.	Positions sketched on field sheet and diameters estimated in 2-inch classes.

⁵ Average height attained by dominant and codominant trees at the age of 50 years.

ANALYSIS OF DATA

In order to analyze factors affecting increase in growth it was necessary to project many values back to the time of release. This operation involved material difficulties. In some cases it was facilitated by the use of detailed stem analyses of 84 sample trees selected at random.

A curve showing diameters inside bark corresponding to given diameters outside bark was set up from the values determined for these sample trees. The diameters inside bark of the 1,464 trees at the time of study were then read from this curve. When the diameters inside bark at different periods had been obtained for the individual sample trees by subtracting twice the radius increment as measured on the increment cores they were translated to diameters outside bark by reference to the same curve.

A curve relating height at time of release to height at time of study was likewise constructed on the basis of these stem analyses, and was used in estimating the heights of the study trees at the time of release.

A curve showing crown ratios⁶ at the time of release corresponding to given crown ratios at the time of study was constructed from the stem-analysis data on the assumption that the height to base of crown of the individual trees had not changed since the time of release, and was used for projecting crown ratios of the study trees back to the time of release.

Estimation of crown width at the time of release was impracticable. Analysis of the growth of the branches on a few selected trees indicated that the horizontal expansion of the crowns was in most cases so small as to be negligible in comparison with the errors inherent in the methods of estimating crown width. Crown width estimated at the time of study was therefore used in the computations as crown width at the time of release.

The next step in the analysis of the data was to find a method of distinguishing, for individual trees, between (1) the growth that would have been made without release, and (2) the additional growth made, i.e., the growth attributable to release. The mean annual basal-area⁷ growth at breast height for the 5-year period before release was taken as the equivalent of the growth that would have been made if the trees had not been released. This basis of comparison insured conservatism in estimates of growth increase due to release, since experience has shown that the rate of basal-area growth for individual trees in the lower crown classes, such as composed the residual stands included in this study, usually decreases and seldom or never increases unless the trees are influenced by changes in stand conditions. Accordingly, the portion of the growth attributable to release was computed for each tree as follows: (Basal area 10 years after release—basal area at time of release) ÷ 2 (basal area at time of release—basal area 5 years before release). The values so obtained are herein referred to as increase in basal-area growth.

The increase in growth is graphically illustrated by the distance between the numerals 3 and 4 on the radius marked in figure 3.

The mean increase in basal-area growth shown by the study trees for the 10-year period following cutting was 0.093 ± 0.00185 ⁸ square

⁶ Crown length in percentage of total height. All values considered in this paper are expressed in decimals.

⁷ Cross-section area in square feet.

⁸ Standard error = $\frac{\text{Standard deviation}}{\sqrt{\text{Number}}}$.

feet per tree, or 130 percent of the total mean basal-area growth which, according to estimates, the trees would have put on in that period if they had not been released. The smallness of the standard error of this mean in relation to the mean shows definitely that most loblolly pine trees left by cutting to an elastic 10-inch diameter limit in stands such as those studied increase in rate of basal-area growth during the 10 years following release. The chances are less than 1 in 1,000,000,000 that the mean basal-area growth of another sample of

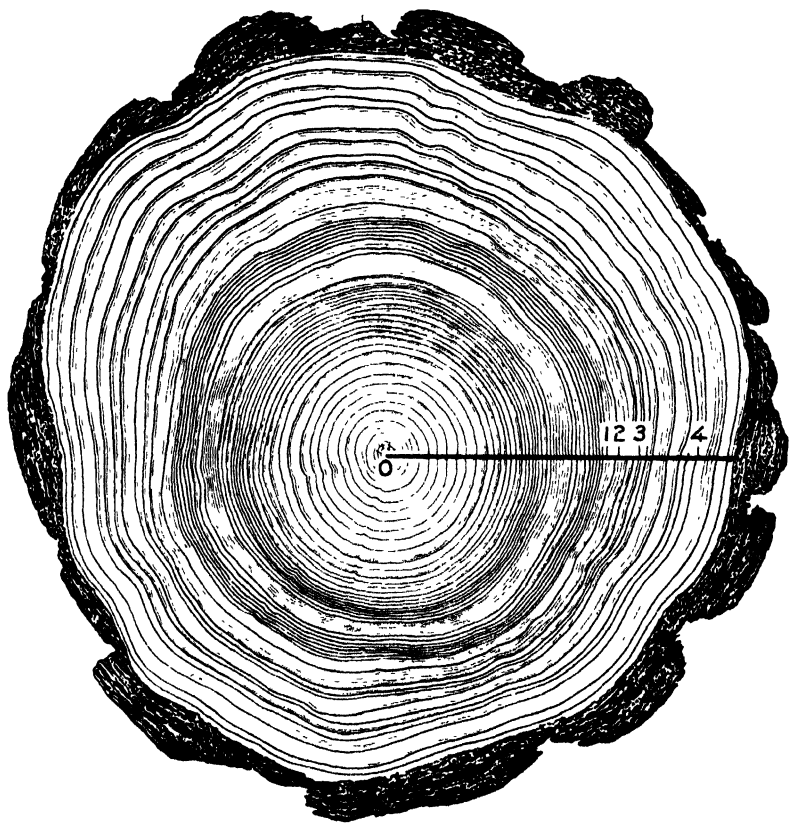


FIGURE 3.—Section of loblolly pine tree cut 12 years after release: 0-1, radius 5 years before release; 0-2 radius at time of release; 0-3, radius 10 years after release if basal-area growth had continued at the same rate prevailing in the 5-year period before release, 0-4, actual radius 10 years after release

1,464 loblolly pine trees growing under like conditions (before and after release) would show no increase after release.

The change in rate of basal-area growth per tree in the 10 years following release varied widely, ranging from -0.10 to $+0.45$ square feet. A cursory examination of the data indicated that this wide variation was correlated in part with variations (1) in characteristics of the trees studied, hereafter referred to as tree factors, and (2) in degree of competition prior to release and degree of release from competition, which for the sake of brevity will be called the competition and release factors.

TREE FACTORS

The tree factors selected for consideration in the analysis were diameter, crown ratio, crown width, total height, and basal-area growth prior to cutting. In the sample studied the trees that had large diameters at the time of release showed less increase in basal-area growth than the smaller trees. The relationship between increase in basal-area growth and diameter was found to be distinctly curvilinear. Translation of diameter into basal area made the relationship linear (fig. 4, *A*).

Long-crowned trees plainly showed greater response to release than those with short crowns (fig. 4, *B*). Likewise, wide-crowned trees showed greater increase than those with narrow crowns (fig. 4, *C*). Trees about 60 feet tall showed greater increase in growth than either taller or shorter trees (fig. 4, *D*); this relationship, however, was not very distinct. No definite correlation was shown between basal-area growth during the 5 years before release and increase in basal-area growth after release (fig. 4, *E*).

It appeared that the factors just listed were intercorrelated and that the correlation of variations in any one of them with variations in increase in basal-area growth, the others being held constant, might be entirely different from the relationships shown in figure 4. Because the sample of trees was so small, it was impossible to sub-sort the data in such a way as to hold several factors constant. Mathematical correlation was chosen as the next best method of analysis, because it provides a measure of the relationship between two factors, others being held constant mathematically at their means.

Accordingly, increase in basal-area growth was correlated with the five tree factors considered in the foregoing, that is, basal area, crown ratio, crown width, and total height at time of release, and basal-area growth during the 5 years before release. The resulting equation was:

Increase in basal-area growth in the 10 years following release equals -0.1397 basal area $+0.2837$ crown ratio $+0.00163$ total height $+0.00633$ crown width -0.3196 basal-area growth during the 5 years prior to release -0.1116 .

When the regression equation is interpreted in the usual way, that is, on the basis that the regression coefficient of any independent variable measures the change in the dependent variable associated with a unit change of that independent variable, the other independent variables being held constant at their means, it appears that the small, tall trees with long, wide crowns, which were growing the slowest before release, usually showed the greatest increase in basal-area growth after release. This conclusion was substantiated by the fact that all the regression coefficients were at least 4.5 times as large as their standard errors, proof that each factor was significantly correlated with increase in basal-area growth when the others were held constant.

When the regression coefficients were translated into β coefficients⁹ and so made directly comparable, it was found that

$$\beta \text{ basal area} = -0.2906$$

$$\beta \text{ crown ratio} = 0.3777$$

$$\beta \text{ crown width} = 0.3736$$

$$\beta \text{ total height} = 0.2408$$

$$\beta \text{ basal-area growth during the 5 years before release} = -0.1151$$

⁹ β = Regression coefficient $\frac{\text{Standard deviation of independent variable}}{\text{Standard deviation of dependent variable}}$.

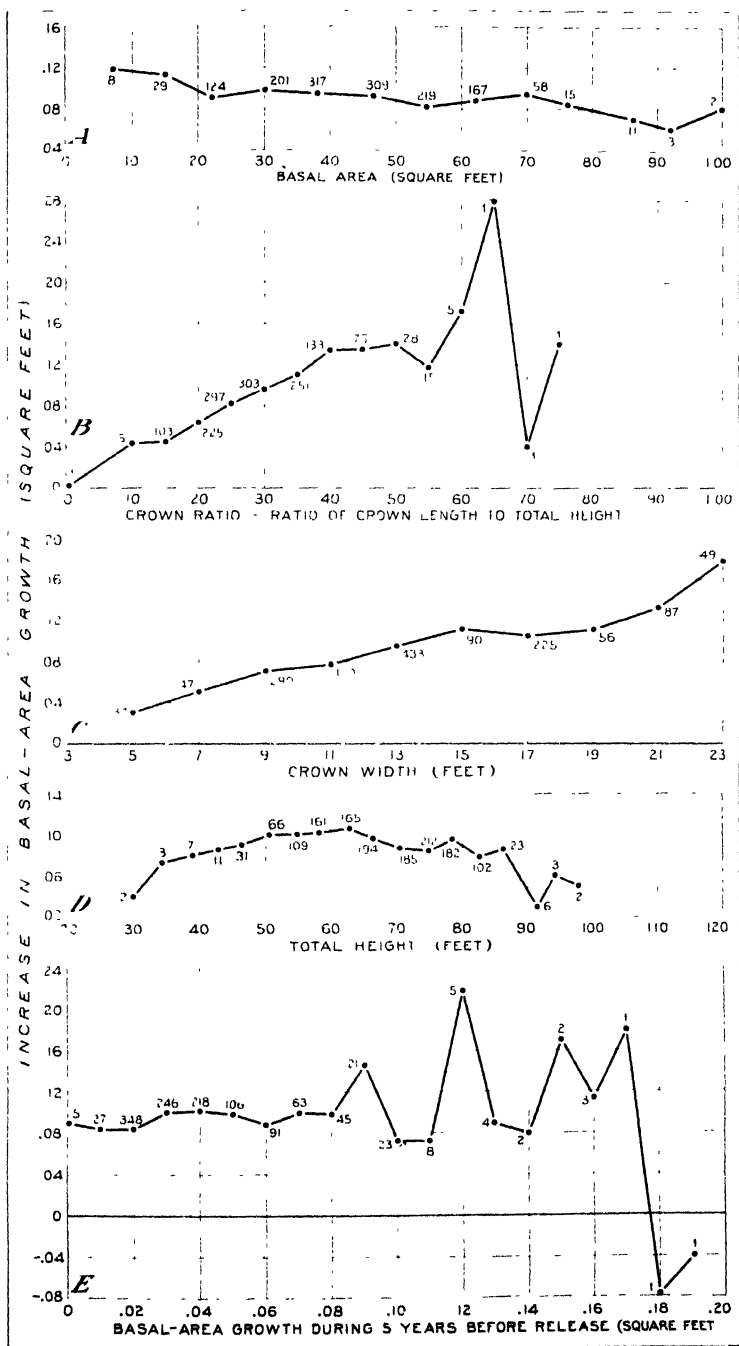


FIGURE 4.—Gross correlations between tree factors at time of release and change in rate of basal-area growth in the 10-year period following release. Numerals indicate number of trees in each class. A, Basal area (square feet); B, crown ratio (percentage of total height); C, crown width (feet); D, total height (feet); E, basal-area growth during the 5 years before release (square feet).

It is plain that, of the five factors considered, crown ratio and crown width had the greatest influence on increase in basal-area growth after release, and that basal-area growth before release had the least influence.

Although no significant gross correlation between total height and increase in basal-area growth was shown (fig. 4, *D*), a marked correlation between these factors appeared when basal area, crown ratio, crown width, and basal-area growth before release were held constant. Basal-area growth before release also showed much more definite correlation with increase in basal-area growth after release when the other tree factors included in the analysis were held constant.

COMPETITION AND RELEASE FACTORS

A consideration of increase in growth following release would not be complete without an attempt to measure the effects of various degrees of release on trees differing in characteristics and previously subjected to various conditions of competition. The first problem encountered in this phase of the work was the selection of measures of competition and release. Any measure or measures of competition should include number and size of competing trees; quantity of tree reproduction, of shrubs, and of herbs; the distance of trees and other competing vegetation from the tree studied; their position in regard to the tree studied and to each other; and their characteristics. No specific data were available on competing tree reproduction, shrubs, and herbs. Data on competing trees were available for all these items except the last from sketches taken for each study tree and its surroundings. It was impracticable, however, to consider in full detail even all of the factors for which data were available. Accordingly, the number and size of competing trees located between 0 and 10 feet, between 10 and 20 feet, and between 20 and 30 feet¹⁰ of the tree studied were chosen as the factors best lending themselves to analysis of the small quantity of data at hand.

Several methods of evaluating these measures were tried and rejected because they were logically inadequate or plainly would not allow the use of the correlation method in the analysis. For example, total basal area of all the trees in each of the 10-foot zones at the time of partial cutting, and percentage of the basal area removed from each of the three zones, were rejected because when analyzed on this basis the data exhibited a definite tendency toward joint correlation.¹¹ Finally it was decided that analysis of competition and release should be based on the number of trees cut, the number of trees left, the average basal area of the trees cut, and the average basal area of the trees left, in each of the three zones.

A multiple correlation of increase in basal-area growth with these factors and the five tree factors previously considered, computed in an attempt to determine in what degree any one of the several factors was correlated with increase in basal-area growth, the others being held constant at their means, gave some results difficult to reconcile with past findings.

As is shown in table 1, the regression equation indicates that a variation of 1 in the number of trees left in the 10-foot zone was not

¹⁰ These ranges of distance are hereafter termed the 10-, 20-, and 30-foot zones.

* ¹¹ Joint correlation is that type in which the degree of correlation between an independent variable and the dependent variable is governed by values of another independent variable.

associated with so much variation in increase in basal-area growth as a variation of 1 in the number of trees (of the same size) left in either the 20- or the 30-foot zone. This indication, which might be interpreted to mean that trees left in cutting suffer less competition from trees left within 10 feet of them than from the same number of trees separated from them by a distance of from 10 to 30 feet, is inconsistent with the general experience of woodsmen.

TABLE 1.—Regression coefficients, and their errors, obtained in multiple correlation of certain variables with increase in basal-area growth of loblolly pine in the 10 years following release

Variable ^a	b ^b	σ_b ^c	t ^d
Basal area of study tree at time of release	-0.1487	0.01530	9.72
Crown ratio of study tree at time of release	+ .2729	.01877	14.54
Total height of study tree at time of release	+ .0016	.00022	7.27
Crown width of study tree at time of release	+ .0075	.00018	15.62
Number of trees left within 10 feet of study tree	- .0006	.00050	1.20
Number of trees cut within 10 feet of study tree	+ .0159	.00252	6.31
Number of trees left 10 to 20 feet from study tree	- .0060	.01380	.43
Number of trees cut 10 to 20 feet from study tree	+ .0032	.00102	3.11
Number of trees left 20 to 30 feet from study tree	- .0018	.00151	1.17
Number of trees cut 20 to 30 feet from study tree	+ .0013	.00080	1.62
Average basal area of trees left within 10 feet of study tree	+ .0067	.01015	.66
Average basal area of trees cut within 10 feet of study tree	- .0031	.00392	.79
Average basal area of trees left 10 to 20 feet from study tree	+ .0108	.00593	1.82
Average basal area of trees cut 10 to 20 feet from study tree	+ .0325	.00329	76
Average basal area of trees left 20 to 30 feet from study tree	+ .0108	.00587	1.81
Average basal area of trees cut 20 to 30 feet from study tree	- .0075	.00391	1.92
Basal-area growth of study tree in the 5 years before release	- .2238	.07338	3.05

^a Constant term is -0.1372

^b Regression coefficient denoting regression of variable on increase in basal area growth, other variables being held constant at their means

^c Standard error of regression coefficient

^d Regression coefficient in terms of its standard error. Figures given in this column show by how many standard errors the regression coefficients are removed from 0. The limit of significance usually accepted is 2 standard errors.

Another relationship inconsistent with general observations is indicated by the coefficients of average basal area of trees cut and of trees left at different distances from the study trees. The coefficients as determined in the equation indicate that the removal of large trees from the 10- or 30-foot zones was associated with less increase in basal-area growth than the removal of large trees from the 20-foot zone.

These inconsistencies suggested that (1) important variables were not considered in the equation, (2) the relationships were curvilinear, (3) the relationships were joint, or (4) the relationships were joint and curvilinear.

Of these possibilities the first was not investigated, because data on other variables were not available. This lack of data was unfortunate, because it cannot be doubted that variations in several other tree factors, and in degree of competition from tree reproduction, shrubs, and herbs, had an important bearing on increase in growth.

The relationships were found to be linear, by the use of the Bruce and Reineke technic (2).

In investigating the third and fourth alternatives it was necessary to sub-sort the data according to different degrees of competition and of release from competition. The fineness of this sub-sorting was limited by the fact that so few data were available. Accordingly, the only competition and release factors recognized were number of trees

cut and number of trees left in the 10- and 20-foot zones. Even in these classes, the data in each group created by subsorting were very scanty. The largest general group, that with 0 trees cut and 0 trees

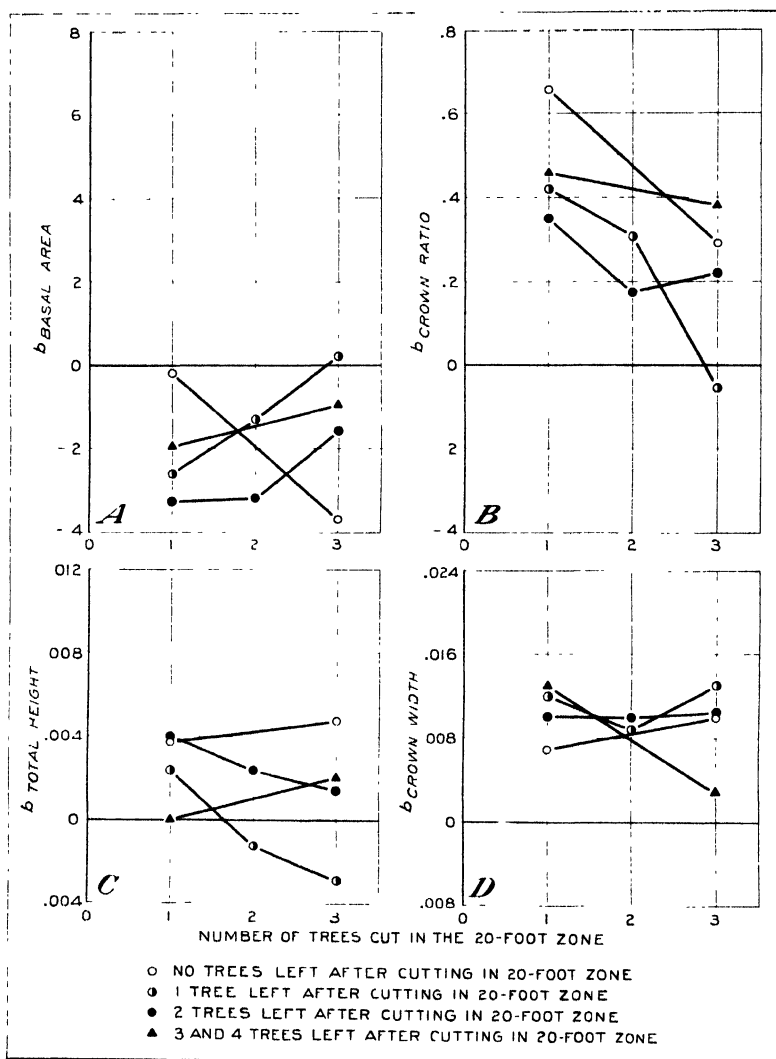


FIGURE 5.—Relations between multiple regression coefficients of basal area *A*, Crown ratio *B*, total height *C*, and crown width *D* as correlated with increase in basal-area growth in the 10 years following release, and degree of release as measured by number of trees cut in the 20-foot zone when different numbers of trees were left following cutting. In all cases no trees were present in the 10-foot zone before or after cutting.

left in the 10-foot zone and with varying numbers of trees cut and left in the 20-foot zone, was chosen for the more detailed analysis. This group was subdivided into groups having constant numbers of trees cut and left in the 20-foot zone.

Table 2 shows the regression equations obtained by correlating increase in basal-area growth with four tree factors for each group.

Figure 5 indicates a possible correlation of the regression coefficients with degree of release as measured by number of trees cut in the 20-foot zone. For the study trees this possible correlation is most pronounced in the case of basal area and crown ratio; even in these cases it is questionable, because of the comparatively large errors of the regression coefficients, shown in table 2.

TABLE 2.—Regression coefficients obtained by correlating increase in basal-area growth in the 10 years following release with basal area, crown ratio, total height, and crown width at time of release of study trees within 20 feet of which varying numbers of trees were cut and left, respectively, no trees being cut or left within the 10-foot zone

Number of trees in 20-foot zone		Characteristics of study trees at time of release ^a									Number of instances
		Basal area		Crown ratio		Total height		Crown width			
Cut	Left	<i>b</i> ^b	<i>σ_b</i> ^c	<i>b</i> ^b	<i>σ_b</i> ^c	<i>b</i> ^b	<i>σ_b</i> ^c	<i>b</i> ^b	<i>σ_b</i> ^c		
1	0	-0.0153	0.1391	+0.6508	0.1914	+0.0038	0.0024	+0.0071	0.0046	22	
2	0										
3	0	- .3747	.1388	+ .2916	.1695	+ .0046	.0022	+ .0097	.0044	34	
1	1	- .2584	.0954	+ .4145	.1486	+ .0024	.0014	+ .0118	.0035	29	
2	1	- .1322	.1160	+ .3106	.1218	- .0012	.0015	+ .0081	.0029	32	
3	1	+ .0159	.1223	- .0531	.1235	- .0025	.0019	+ .0131	.0032	40	
1	2	- .3283	.0952	+ .3534	.1683	+ .0039	.0025	+ .0104	.0036	25	
2	2	- .3243	.1238	+ .1784	.1607	+ .0024	.0022	+ .0097	.0048	25	
3	2	- .1606	.2397	+ .2161	.2484	+ .0014	.0033	+ .0106	.0046	24	
1	3 or 4	- .1978	.0916	+ .4760	.1608	+ .0004	.0016	+ .0131	.0038	31	
2	3 or 4										
3	3 or 4	- .0935	.0663	+ .3887	.0908	+ .0019	.0011	+ .0029	.0022	30	

^a Independent variables.

^b Regression coefficient denoting regression of independent variable on increase in basal-area growth, other independent variables being held constant at their means

^c Standard error of regression coefficient.

If these relationships are true, the degree of correlation between basal area at time of release and increase in basal-area growth after release tends to approach 0 as the degree of release is increased, competition before release and tree characteristics being constant. Likewise, such relationships might indicate that the degree of correlation between crown ratio and increase in basal-area growth approaches 0 as the degree of release is increased. No evidences of curvilinear relationships were found in these several equations.

PREDICTION MECHANISMS DEVELOPED, AND THEIR APPLICABILITY

Analysis of the data available in this study has resulted in development of the following equations for predicting the sizes of individual loblolly pine trees 10 years after release:

(1) $0.8681 \text{ basal area (including bark) at time of release} + 0.2914 \text{ crown ratio} + 0.0065 \text{ crown width} + 1.6332 \text{ basal-area growth during the 5 years before release} + 0.0017 \text{ total height} - 0.1212 = \text{basal area (including bark) 10 years after release.}$ Standard error of estimate = 0.0613 square foot.

(2) $1.0332 \text{ basal area (including bark) at time of release} + 0.3116 \text{ crown ratio} + 0.0088 \text{ crown width} - 0.0602 = \text{basal area (including bark) 10 years after release.}$

bark) 10 years after release. Standard error of estimate = 0.0726 square foot.

(3) 0.9564 basal area (including bark) at time of release + 2.2872 basal-area growth during the 5 years before release + 0.1015 = basal

Key A to D, hold E; to F, read C

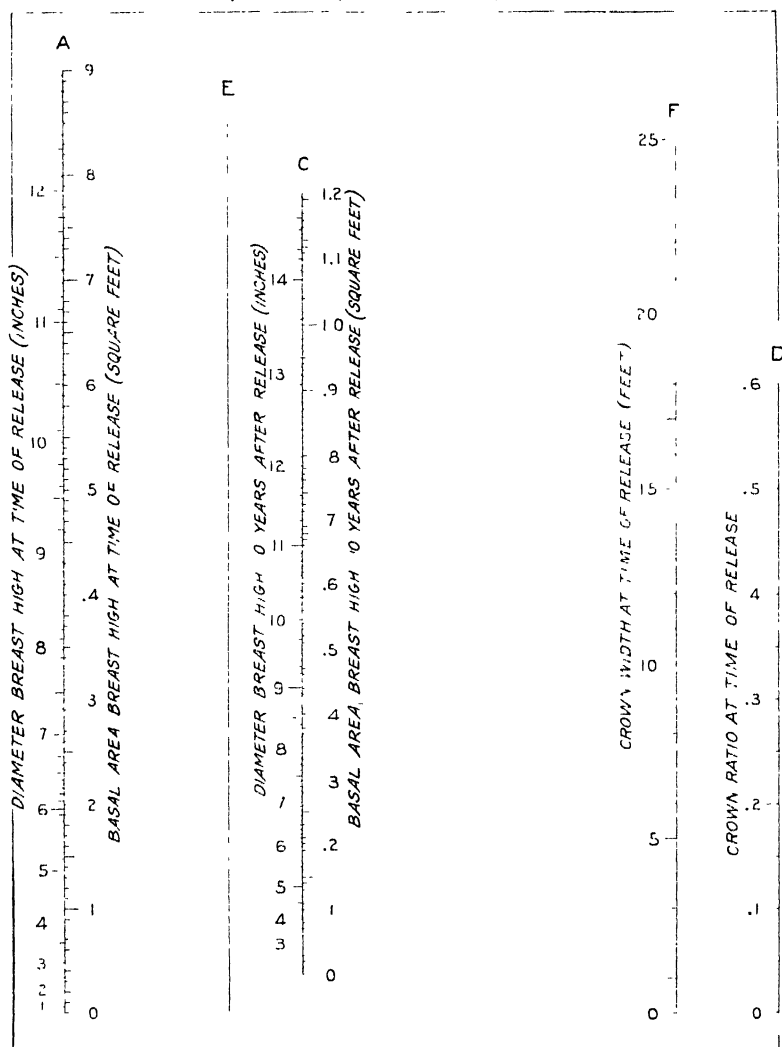


FIGURE 6.—Alignment chart for predicting sizes of individual trees 10 years after release by partial cutting. The chart is based on the equation 1.0332 basal area (including bark) at time of release + 0.3116 crown ratio + 0.0088 crown width - 0.0602 = basal area (including bark) 10 years after release.

area (including bark) 10 years after release. Standard error of estimate = 0.0708 square foot.

A consideration of the standard errors of estimate for these three equations shows that (1) is the most accurate as a predicting mechanism and that (3) is second in accuracy. For practical purposes equations (2) and (3) are more usable than equation (1), and they are therefore presented in alignment-chart form in figures 6 and 7.

The use of either of these predicting mechanisms requires caution. It must be remembered that they will give results within the standard errors presented only if they are applied to trees growing in stands

Key: A to B, read C

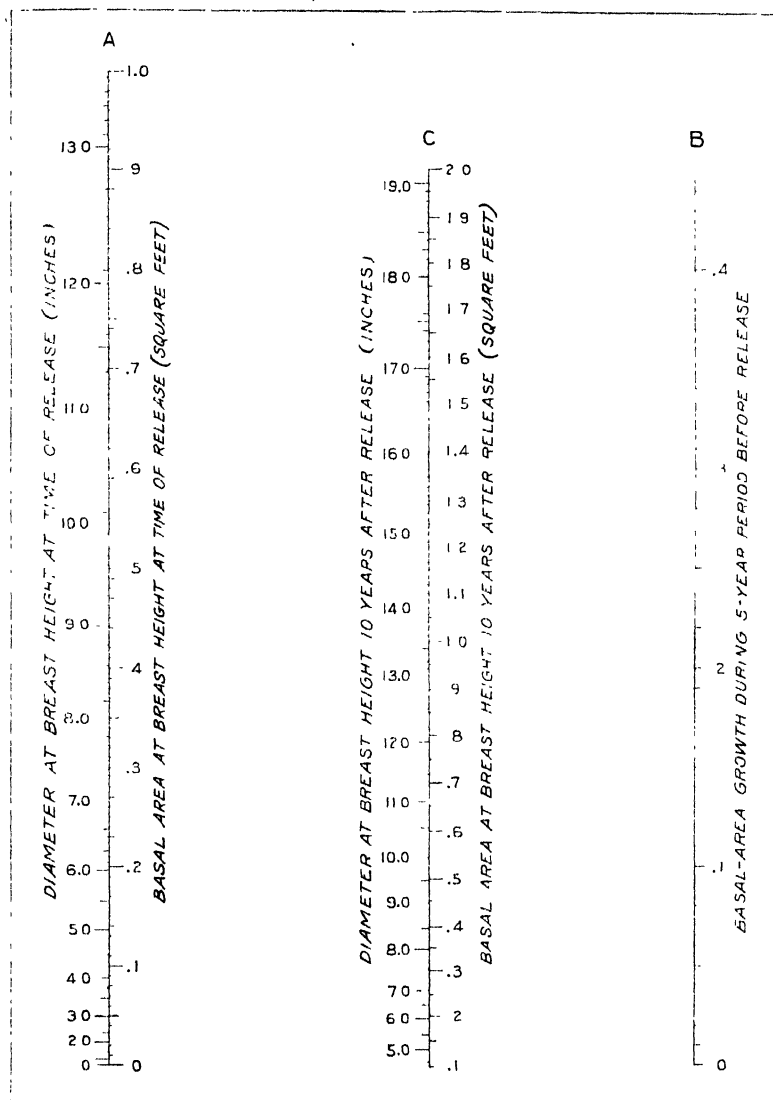


FIG. 7.—Alignment chart for predicting sizes of individual trees 10 years after release by partial cutting. The chart is based on the equation $0.9564 \text{ basal area (including bark) at time of release} + 2.2472 \text{ basal-area growth during the 5 years before release} + 0.1015 = \text{basal area (including bark) 10 years after release}$

similar to those included in this study and only if the stands have been or are to be cut to an elastic 10-inch diameter limit and the distribution by size classes of trees cut and trees left approximates that presented in figure 2.

It does not appear that these limitations will greatly restrict the use of the equations on stands cut over under present cutting prac-

tices. Most of the loblolly pine stands that are now being cut are between 40 and 65 years old. Likewise, most of them are between 50 and 70 percent stocked by basal area and are growing on areas with site indices between 80 and 90 feet. In present cutting practice the elastic 10-inch diameter limit is the prevailing standard. If the equations are to be applied under future conditions, however, it will probably be necessary to extend the study to include areas cut over to other diameter limits. The probability that revision will be needed to meet future conditions is particularly great as regards application of the equations to areas cut over for lumber, since recent studies (6, 7) have shown that in lumber operations the removal of trees less than 12 inches in diameter breast high is in many cases unprofitable.

The apparent variations in the correlation of tree factors and growth increase on the one hand with different degrees of release on the other led to the conclusion that with the small quantity of data at hand the effects of different degrees of release on growth increase could not be satisfactorily analyzed.

From the regression equation including the five tree factors as independent variables the tentative conclusion may be drawn that in order to get the greatest basal-area increase per tree after release the small, tall trees with long, wide crowns should be left in cutting.

SUMMARY

The basal-area growth at breast height of loblolly pine trees released by cutting to an elastic 10-inch diameter limit was found to be 130 percent greater, on an average, in the 10 years following release than the growth which, according to estimates, the trees would have put on in that period had they not been released.

Variations in the growth increase of the individual trees in the stands studied were shown to be correlated in part with variations in individual tree characteristics. Crown ratio, crown width, basal area, and total height at the time of release, and basal-area growth during the 5-year period prior to release, were shown to be correlated with increase in basal-area growth after release, in degrees diminishing in the order named.

Measurement of the correlation between degree of competition prior to release and degree of release from competition on the one hand and increase in growth following release on the other was difficult because of the complexity of the relationships involved, including tree-factor relationships. No satisfactory method was found, in fact, for measuring the effects of different degrees of release on the growth of trees having the same characteristics.

Mechanisms were developed for predicting the sizes of individual trees 10 years after release. These are theoretically applicable only to trees growing under the same general conditions as the trees included in the sample, and to stands cut in approximately the same way as those sampled. Most of the loblolly pine stands now being cut in the Atlantic coastal plain are, in fact, highly similar in age, density of stocking, and size to the stands in which the study trees were growing prior to release.

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THE TIME FACTOR IN FERTILIZATION AND EMBRYO DEVELOPMENT IN THE SUGAR BEET¹

By ERNST ARTSCHWAGER, *pathologist*, and RUTH C. STARRETT, *assistant cytologist*,
Division of Sugar Plant Investigations, Bureau of Plant Industry, United States
Department of Agriculture

INTRODUCTION

Although investigations during the past decade have conclusively shown that the embryo sac of the sugar beet (*Beta vulgaris* L.) is mature at the time of anthesis and that the pollen is viable and the stigma receptive even before the anthers dehisce, the time factor in fertilization and embryo development has remained a debatable issue; therefore, it was the purpose in this investigation to study, on a large amount of material, the sequence of the nuclear phenomena in the embryo sac from the time of anthesis to the development of the embryo.

MATERIAL AND METHODS

The material for study was obtained from a 10-acre field of mother beets of the Flat Foliage variety grown on the farm of the American Beet Sugar Co. at Rocky Ford, Colo. Practically all the flowers were collected during the height of the blooming period between the 8th and the 20th of June in 1931 and 1932. Several hundred plants were selected and marked by numbered stakes. On each of these plants from 5 to 15 flowers were marked. The location of these flowers was indicated by a small numbered tag placed around the branch of the floral axis near the flower cluster in question. The flower cluster itself was marked by placing an india-ink dot on the subtending bract. In every case the oldest flower of a cluster was chosen principally to avoid mistakes in collecting. Flowers were usually marked in the morning, since at that time anthesis is most general, and collections were made at intervals of several hours, the latest collection being made 14 days after anthesis. No later collections were found necessary, since in material 12 to 14 days old the embryos are mature.

The material marked for study was separated with a sharp safety-razor blade from the other members of a flower cluster and dropped immediately into a vial with fixing solution. Most flowers were killed in Carnoy's solution, but a few hundred of the 1932 collection were fixed in a modification of Nawaschin's solution to bring out more clearly the location of the pollen tubes. The larger amount of the material was embedded in paraffin after being taken through the xylol-alcohol series. The results obtained were not inferior to those with the butyl alcohol technic, although the latter reagent somewhat facilitated the cutting of flowers with old embryos.

All cytological material was stained with Heidenhain's iron-alum haematoxylin. The photomicrographs were taken on Wratten and Wainwright M plates with B-58 and E-22 filters used singly or in combination. The drawings² are based on photomicrographs.

¹ Received for publication July 7, 1933; issued February, 1934.

² Credit is due Mrs. E. Artschwager for the preparation of all drawings in this paper.

MORPHOLOGY OF THE BEET FLOWER

The small sessile flowers of the sugar beet are usually borne in clusters of two or more which are attached to the inflorescence axis or secondary branches of the latter. The flowers are perfect; the calyx is 5-parted and incurved, adhering to the base of the ovary and becoming hard in the fruit. There are five stamens. The 3-carpellary ovary is sunk in a fleshy disk enclosing a campylotropous ovule which is attached laterally to the ovary wall by a short funiculus. The style is very short, terminating in a 3-, sometimes 4- or 5-lobed stigma which persists in the fruit.

While most beet flowers, under Colorado conditions, open soon after 7 a.m., anthesis continues throughout the day. Similar observations are recorded by Oksijuk (6)³ for the beet-growing regions of Russia. As the flowers open, the sepals either expand completely to form an angle of 90° with the floral axis or they open up only partially. In either case the sepals close again more or less completely (fig. 1, *E, F*) after embryo development is initiated. As the sepals begin to expand the anthers open by longitudinal fissures. The fresh pollen grains at first adhere together in clumps, but these soon dry and the pollen is carried by wind and insects to the receptive stigmas of other flowers. A blooming beet field is alive with visiting insects, and since in the morning hours the atmosphere is usually calm, air currents may often be only of minor importance in the transfer of pollen from flower to flower and from plant to plant.

OPENING OF STIGMA LOBES

At the time of anthesis the stigma has the form of an erect conical tube (fig. 1, *A*) composed of a number of folded lobes. In the majority of flowers this number is 3; not infrequently, however, there are 4 (fig. 1, *B, C, D*) and even 5. In such cases the expanded stigma is not symmetrical, as some of the lobes are larger than others. Van Heel (4) also reported the occurrence of multilobed stigmas in some of his material. The stigma lobes begin to unfold some time after the flower has opened; this process is a gradual one, and the time necessary for complete expansion varies with individual flowers. According to early writers on sugar-beet breeding, the stigma begins to unfold the day after flowering and the stigma lobes are fully expanded only after the third day. Recent observations reported by Oksijuk (6) place the first indication of unfolding at 5½ hours after anthesis, with the entire process completed in 24 hours.

Some data on the opening time of the stigma lobes under Colorado conditions are reported in table 1. Of 54 flowers examined 7 hours after anthesis, about one fourth had stigma lobes still closed. Another lot of 61 flowers, examined after 24 hours, showed most stigmas at various stages of unfolding, with only a small percentage remaining unchanged. A final observation made 36 hours after anthesis showed practically all flowers with stigma lobes fully expanded. Earlier results obtained in 1931 (but not recorded in table 1) indicate that the stigma lobes of some flowers started to open as early as 5 hours after anthesis and had completely expanded several hours later. In general, however, the stigma begins to unfold in midafternoon, or about 7 hours after the flowers open, and expansion is completed

³ Reference is made by number (italic) to Literature Cited, p. 843.

within 36 hours. Just as the sepals of a beet flower occasionally fail to fold back completely, so the stigma lobes at times only partially

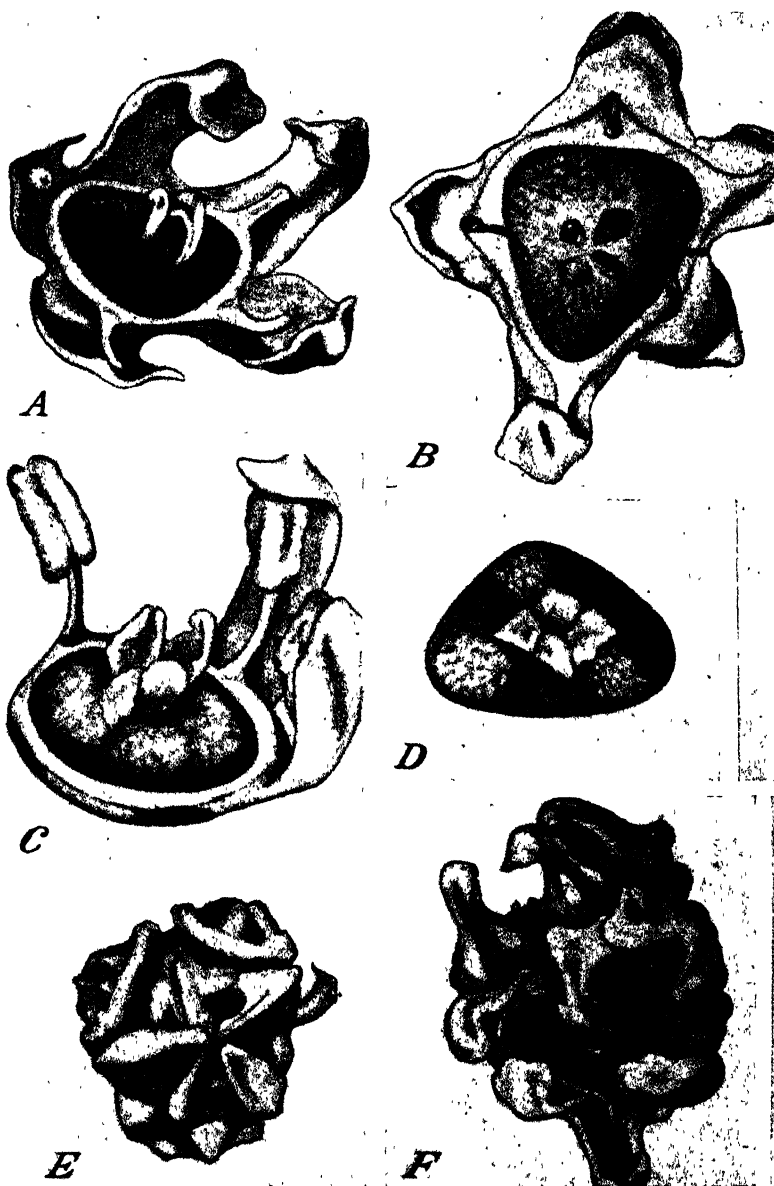


FIGURE 1.—A, Young beet flower with anthers removed, seen from above, the stigma lobes just beginning to expand; B, 3-day-old flower with stigma lobes fully expanded; C, 1-day-old flower with stigma lobes partly expanded; D, 2-day-old flower with stigma lobes fully expanded; E, partly mature seed ball with sepals folded tightly; F, partly mature seed ball with sepals folded back only partly.

expand. This latter condition is not infrequent and may be the result of early growth of the pollen tubes, although observations supporting such a correlation have not as yet been made.

TABLE 1.—*Opening of the stigma lobes*

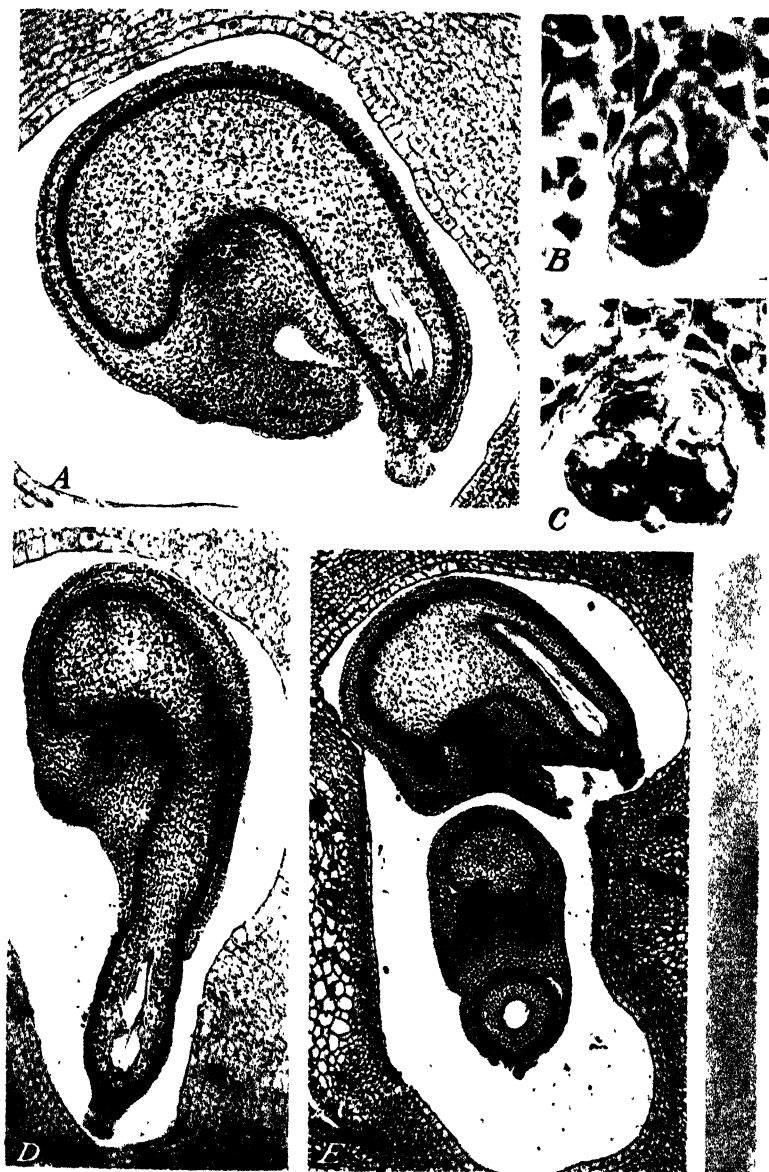
Time after anthesis	Plant no.	Flowers examined	Flowers with stigma lobes—			
			Closed	One fourth open	One half open	Open
7 hours.....	1	4		4		
	2	10		10		
	3	5		5		
	4	4		4		
	5	3		3		
	6	2		2		
	7	5	3	2		
	8	5	3	2		
	9	5	5			
	10	3			3	
	11	3		1	2	
	12	3	3			
	13	2		2		
Total.....		54	14	35	5	
Percent.....			25.9	64.8	9.3	
24 hours.....	14	4			1	3
	15	5			5	
	16	5			5	
	17	4			4	
	18	3			3	
	19	5	2	3		
	20	5	1	4		
	21	5		4	1	
	22	4				4
	23	3			2	1
	24	2			2	
	25	2	1	1		
	26	2		2		
	27	5		5		
	28	2		1	1	
	29	2		1	1	
	30	3			2	1
Total.....		61	4	21	27	9
Percent.....			6.5	34.4	44.3	14.8
31 hours.....	31	4	3	1		
	32	5		2	3	
	33	5			3	2
36 hours.....	34	5				5
	35	5				5

ORGANIZATION OF EMBRYO SAC AT TIME OF FLOWERING

The solitary ovule of the beet flower is campylotropous and lies horizontally in the cavity of the ovary, with its largest diameter at the time of anthesis a little over 0.5 mm. The micropylar tip of the ovule is usually bent slightly upward and often touches the roof of the ovary cavity some distance from the opening of the stylar canal.

The embryo sac at the time of anthesis is a narrow, slightly curved cavity consisting of the embryo sac proper and the caecum (pl. 1, *A*). The latter begins to form before the flower opens, and grows in length at the expense of the nucellar tissue. At the time of anthesis the caecum is as long as the embryo sac proper, but after fertilization it enlarges rapidly, forming a horseshoe-shaped cavity, and attains its final size when the embryo is still very small.

The embryo sac contains the normal nuclear complex. The egg apparatus at the micropylar end of the sac is composed of three more or less pear-shaped cells, the egg and the two synergids (pl. 1, *B*, and fig. 2, *A*). The nucleus of the mature egg usually rests in the basal part of the egg cell and is surrounded by dense cytoplasm. Below the egg nucleus, at the micropylar end, there is commonly present a



A, Horizontal section through ovule at time of anthesis. $\times 94$. *B*, Egg and synergid at time of anthesis. $\times 560$. *C*, Two eggs in embryo sac. $\times 700$. *D*, Ovule with abnormally elongated nucellar tip. $\times 45$. *E*, Two ovules in a single ovary cavity. $\times 45$.

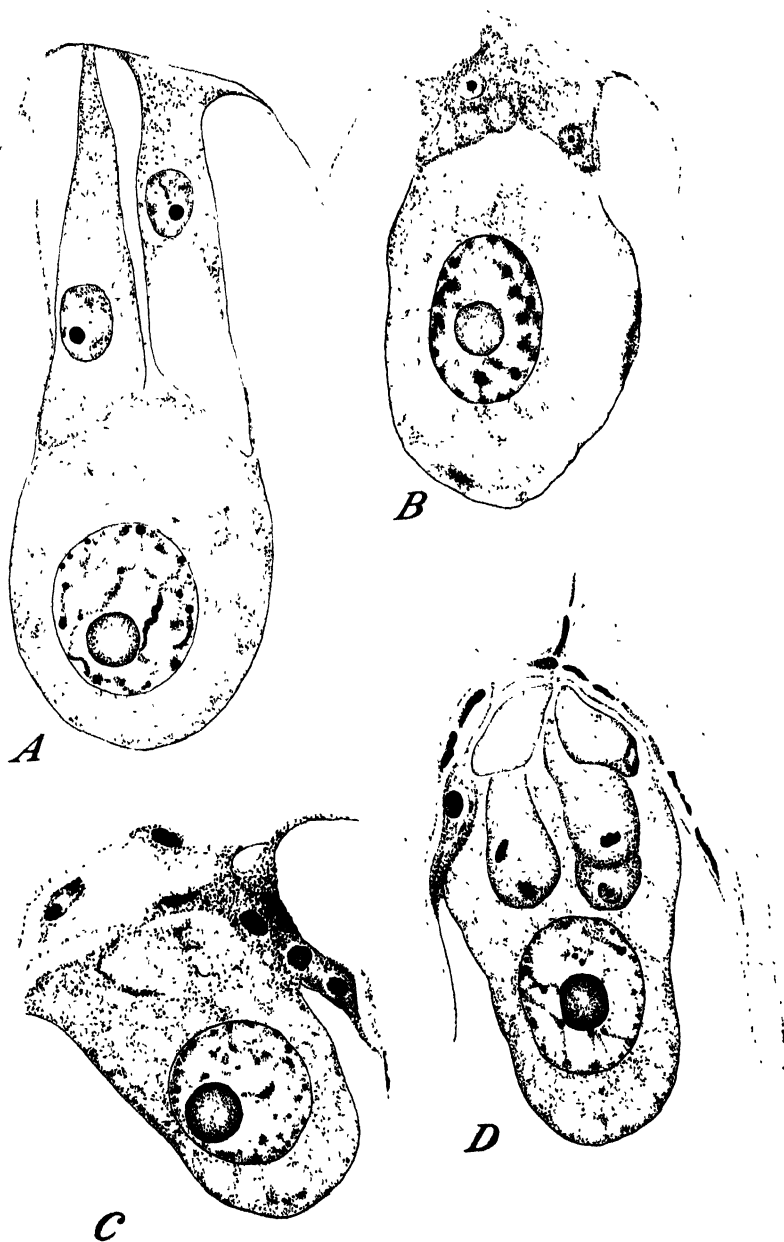


FIGURE 2.—A, Egg with synergids at time of anthesis, $\times 1,440$; B, egg with synergids degenerating, $\times 1,440$; C, fertilized egg with a number of X-bodies, $\times 1,440$; D, egg with pollen tubes and degenerated synergids, $\times 1,440$.

large vacuole. The synergids are slightly shorter than the egg, very slender, and contain large vacuoles near the base; their nuclei are small. There are commonly three antipodals at the chalazal end of the sac. This number is often augmented, especially after anthesis, and frequently an antipodal cell may contain two nuclei. Both Favorsky (3) and Oksijuk (6) observed this multiplication of antipodals, but the latter believed this phenomenon to be uncommon.

The fusion polar or primary endosperm nucleus is very large, and its nucleolus is even more conspicuous than that of the egg. Its position in the embryo sac varies in relation to the antipodals. Of 26 flowers examined at the time of anthesis, 19 showed the fusion polar between the egg and the antipodals, tending rather toward the antipodals; in 2 cases the fusion polar was found opposite the antipodals, and in 5 cases in the caecum below the antipodals.

ABNORMALITIES IN DEVELOPMENT OF OVULE AND EMBRYO SAC

Although the ovary with its ovule and embryo sac develops normally in most flowers, one meets now and then with abnormalities, which, however, are of no practical significance in seed production.

The phenomenon of the development of 2 ovules in 1 ovary cavity (pl. 1, *E*) actually constitutes a coalescence of 2 adjacent flowers, as there is a partial or complete doubling in the number of stamens and stigma lobes, associated, however, with 1 common stylar canal and a single ovary cavity. The two ovules have a normal appearance and may well enough develop into viable seed, although seed balls enclosing two seeds within a common cavity have never been observed.

The occurrence of 2 eggs in an embryo sac (pl. 1, *C*) has been observed only once, and since only 1 synergid accompanied the 2 eggs, it seems quite evident that the supernumerary egg is the sister nucleus of the synergid.

Abnormal elongation of the proximal part of the nucellus (pl. 1, *D*) is sometimes observed. While the inner integument of such ovules accompanies the nucellar protuberance, the outer one ceases growth after having attained its normal development. The embryo sac of such abnormal ovules appears unaffected, and the nuclei are found in their respective positions and, on the whole, show a normal structure.

Suppression of embryo-sac development is not uncommon. The ovules, though normal in appearance, are somewhat dwarfed. The embryo sac is either entirely wanting or is represented by a narrow cavity containing several nuclei.

Favorsky (3) reports the occurrence of polyembryony, a phenomenon never observed in the material on which this investigation was based.

POLLEN

SIZE AND SHAPE OF GRAINS

Pollen grains from a given flower, though variable in size, are fairly uniform, as grains of extreme dimensions are numerically few. Oksijuk (6) noted in his material two types of pollen, namely, a large percentage of small grains of an average diameter of 16.8μ and a comparatively smaller amount of large pollen with a diameter of 21.5μ . He emphasizes the coexistence of these two types, pointing out that beet races differ not only in their gross morphology but

possibly also in their pollen, and that in seed beets, which commonly represent a mixture of races, the parental pollen types have preserved their identity. The writers' data do not harmonize with those of Oksijuk, since only the plotted measurements of *Beta maritima* × Pioneer hybrid (fig. 3, *B*) show a somewhat bimodal distribution. Different beet races show consistent and even significant differences in the average size of their pollen, and it is not impossible that data on

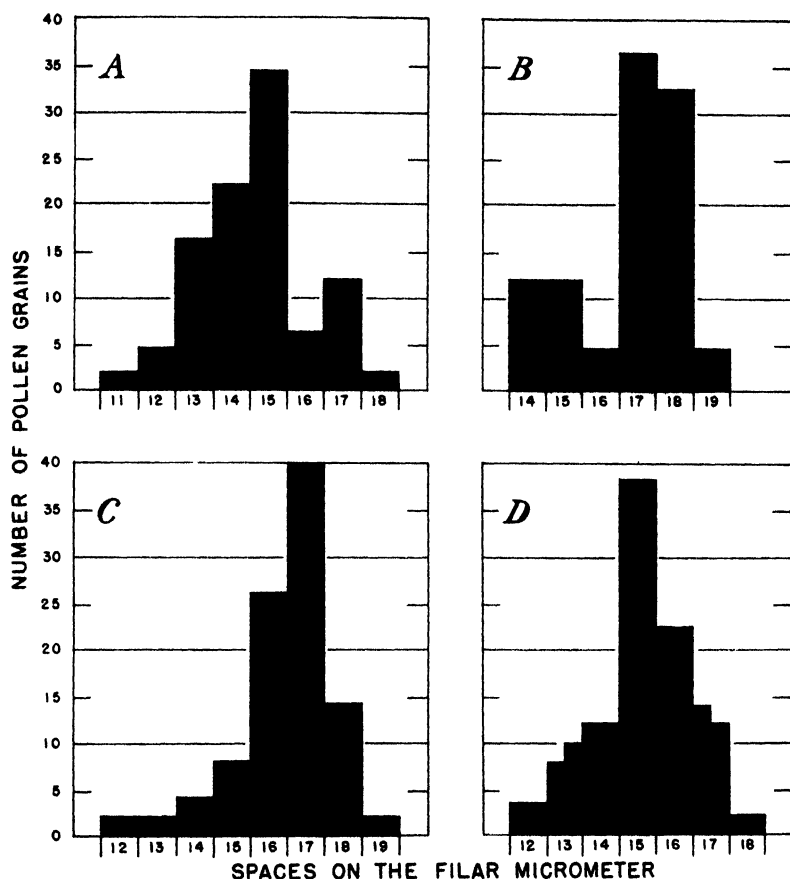


FIGURE 3. Diameter of 100 pollen grains in each of four sugar-beet selections. Each space on the micrometer is 1.42 μ . A, Selection no. 10277-O; B, selection no. 10274-O; C, selection no. 10271-O; D, selection no. 10270-O.

size of pollen could be utilized as an auxiliary diagnostic character in a taxonomic study of beet races.

The mature pollen grain is spherical, its outer membrane thick and sculptured, with numerous thin spots for the exit of pollen tubes. The microspore nucleus divides several days before the anthers dehisce, and gives rise to the tube nucleus and the two generative nuclei. The tube nucleus is more or less spherical with a small nucleolus and dense cytoplasm; the generative nuclei or sperms (pl. 2, *D*) are slender elliptical bodies with chromatic granules em-

bedded in cytoplasm. Young but mature pollen contains a large number of irregular starch grains, but before anther dehiscence the typical iodine reaction is negative, the starch having been changed to sugar.

GERMINATION

Since beet pollen was reputed to germinate poorly on artificial media, germination tests were made on the unopened stigma of dehiscing flowers. For this purpose flowers were marked early in the morning, bagged, and examined in groups at 1-hour intervals. Both cotton blue and *Nachtblau* were found to be selective stains for the pollen tubes of sugar beet, especially if preceded by a short application of acetocarmine. The first germinating pollen grains were observed 2 hours after anther dehiscence. The pollen tubes were short, hardly exceeding the diameter of the pollen grain. Later examinations showed pollen tubes of increasing length with their tips already buried between the papillae of the stigma lobes.

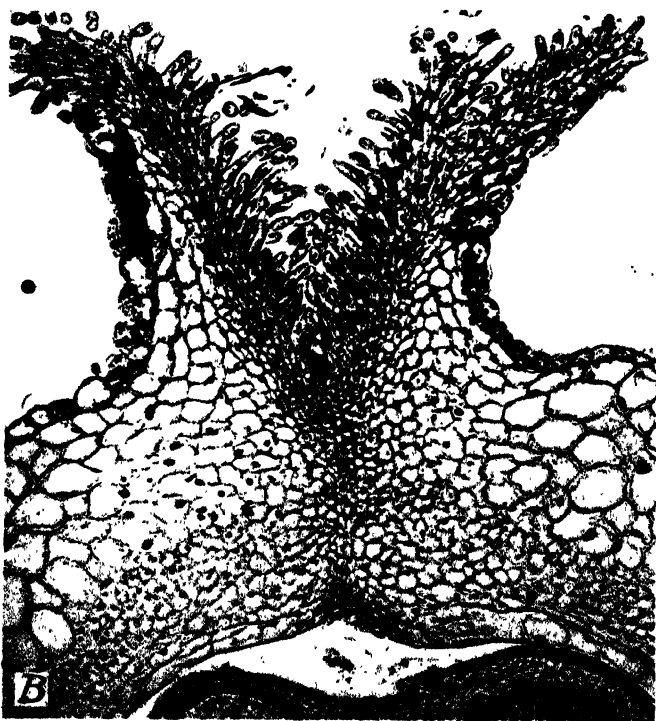
Favorsky (3) attempted to germinate beet pollen on artificial media, obtaining, after numerous trials, best growth on sugar agar of high concentration. Only pollen from old anthers developed pollen tubes, and even then not more than 30 percent of the pollen germinated. Fresh pollen from dehiscing anthers showed scant germination, while mature pollen from unopened anthers did not germinate at all.

In the writers' experiments, carried on in New Mexico and Colorado, pollen was found to germinate easily and abundantly on culture media containing 1½ percent agar and 40 percent sucrose (pl. 2, A). The optimum germination temperature was about 32° C., which corresponds closely to the prevailing day temperatures during the blooming period. Beet pollen may germinate almost immediately, the tubes attaining a length of about 100 microns during the first 2 hours. The growth increment is uniform, though a small number of pollen tubes are commonly well in advance while some few lag behind and cease growth altogether. This differential rate is even more pronounced in young and in very old pollen. Collections made during the morning hours germinated almost as readily and abundantly as old pollen, thus weakening a popular belief that selfing of beet flowers may be hindered through an enforced rest period in freshly shed pollen. These tests showed also that there is a great variation in the germination percentage of pollen of different flowers; in fact, the pollen of some flowers failed to germinate at all.

Favorsky (3), who even under optimum conditions obtained relatively poor germination, tried to arrive at a satisfactory explanation of this condition. He made an extensive study of pollen development and found that a large percentage of pollen degenerated in the later stages of its development. This degeneration seemed to affect all the grains of an anther, and the manner of degeneration frequently varied. He noted particularly that the pollen of otherwise normally stained preparations appeared very dark and that additional destaining resulted in a uniform loss of color without differentiation. The nuclei of such grains were crescent-shaped with frequently only the nucleolus showing. Often the pollen grains appeared quite empty and more or less collapsed, having the appearance of an indented rubber ball. Although partial and even complete degenera-



A, Germinating pollen on artificial medium containing 1½ percent agar and 40 percent sucrose. $\times 200$. *B*, Degenerated and normal pollen; in the latter the sperms and the tube nucleus are visible. $\times 1,050$. *C*, Sterile pollen with nucleus undivided. $\times 1,050$. *D*, Drawing of mature pollen grain showing sperms, tube nucleus, and pollen grains. $\times 2,400$. *E*, Longitudinal section through tip of anther showing degenerated pollen. $\times 510$.



A, Fertilized egg with two endosperm nuclei, one of which is near the egg while the other is in the caecum. $\times 107$. B, Longitudinal section of pistil; the stigma lobes are partly expanded. Note the stigma papillae. The latter as well as the epidermal cells of the style contain numerous protein crystalloids. $\times 120$.

tion of anthers had been noted (2), observations on general degeneration phenomena of sugar-beet pollen had not been made, and therefore the data of Favorsky (3) are of special interest to the writers. Degeneration stages such as he described are illustrated in plate 2, *B*, *C*, and *E*. Such abnormal pollen was found in almost all anthers, and in some flowers degeneration was complete. This rather frequent occurrence of abnormal pollen, while at times perhaps seriously affecting autogamy in beets, should be of little significance in seed production, since relatively enormous masses of pollen are available during the height of the blooming period.

Beet pollen may remain viable for a long period; in fact, the researches of Kharechko-Sawitzkaja (5) indicate that some pollen will germinate after 50 days if kept at low temperature and low humidity. Under field conditions in Colorado the viability of pollen does not extend beyond a day, since beet pollen cannot stand wetting by dew. Where pollen is exposed to the rays of the high-altitude sun the viability seems to depend on the nature of the substratum on which the pollen from dehiscing anthers lodges. Thus, pollen collected on shallow glass dishes, paper trays, and detached beet leaves lost its vitality often in less than 3 hours, whereas pollen resting on living beet leaves retained an undiminished germination energy during the course of a day and the failure to germinate the next morning was probably occasioned by the precipitation of moisture on the leaves.

GROWTH OF THE POLLEN TUBE

The style of the sugar-beet flower is very short and terminates with a three-lobed stigma that is closed but begins to open approximately 5 hours after anthesis. The stylar canal is open and densely clothed with papillate cells. Similar cells constitute the epidermis of the stigma lobes (pl. 3, *B*) which become covered with a sweet secretion, an ideal medium for pollen-tube growth. The plastids of the stigmatic papillae contain protein crystalloids, and similar bodies are found grouped around the nuclei of the cells forming the inner lining of the ovary cavity. Favorsky (3) found, in addition, protein crystals in the epidermis of the sepals and stamens. These bodies are hexagonal, thereby differing from the crystalloids of the stigmatic papillae as seen in plate 2, *E*.

Pollen was thought to germinate only on open stigmas (1), but some observations by Van Heel (4) and the germination studies reported above indicate that some pollen at least will germinate hours before the stigma lobes begin to expand.

Beet pollen germinates with a single pore. The pollen tube grows on the surface of the stigma between the loosely connected epidermal papillae. After penetrating the short stylar canal it extends along the inside of the ovary wall until it reaches the micropyle of the ovule, and thence between the integuments and the intervening layers of specialized nucellar tissue. The cells of the nucellus which are in direct line with the integumental canal are narrow, elongated, and rich in cytoplasm, offering seemingly but little resistance to the growing pollen tube.

The number of pollen grains germinating on an open stigma is large, although much pollen degenerates before anthesis and many pollen tubes grow down the stylar canal into the cavity of the ovary.

Sections through 1-day-old flowers show a tangled mass of pollen tubes near the micropyle, the tips of some of them already in the integumental canal (pl. 4, *E*). Oksijuk (6) reports that he rarely found pollen tubes in the ovary cavity. This may be true of material fixed in Carnoy's solution, but in chromoacetic formalin the tubes as a rule stand out conspicuously. The number of pollen tubes growing down the micropyle varies and as a rule more than one reaches the embryo sac. During early pollen-tube growth the three nuclei remain in the pollen grain but move into the tube only after the latter has attained a given length. The tube nucleus only slightly precedes the sperms, contrary to the findings of Oksijuk (6), who reports that the generative nuclei lag some distance behind the vegetative nucleus.

Upon entry in the embryo sac the pollen tubes apply themselves close to the egg. Their tips are usually dilated and contain the two sperms embedded in densely granular cytoplasm (fig. 2, *D*). The sperms are slightly rounded and have granular chromatin, thus changing from their former elongated, slightly wavy form in the tube (pl. 4, *C*).

FERTILIZATION

ASSOCIATED PHENOMENA

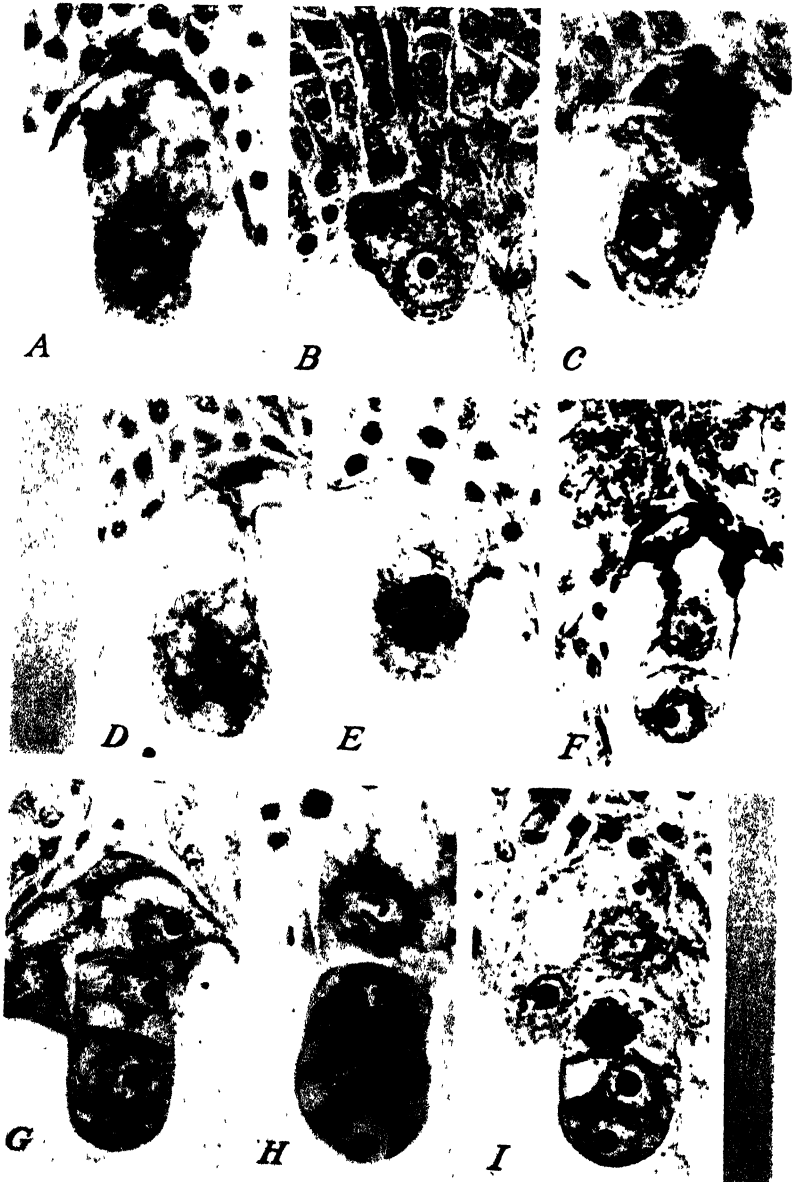
While sperms were observed inside the egg cell in a number of preparations, a study of over 500 fertilization stages failed to show actual fusion between sperm and egg nucleus, which, it appears, is accomplished very rapidly. As a characteristic accompaniment of fertilization there are certain dark-staining round or oval bodies in the vicinity of the egg. These bodies often resemble in form and structure the generative nuclei, and their frequent position in the proximal end of the supernumerary pollen tubes suggests their sperm nature. Most frequently they occur in pairs or double pairs flanking the egg and occupying the place of the degenerating synergids (pl. 5, *B*, *C*). Similar structures have been observed coincident with fertilization in other plants (?). They have been called X-bodies by different writers who interpreted them as the nuclei of the synergids, the degenerated cytoplasmic remains of the male cells, or as the tube nucleus. In the sugar beet these bodies frequently have typical sperm structure even though in older material they will have lost their inner differentiation and appear when stained as homogeneous black bodies. Usually they differ conspicuously from the nuclei of the synergids and only in some preparations might their degenerated remains be interpreted as a part of these so-called X-bodies. These bodies, whatever their nature, persist for a long time and may be easily identified in material containing 10-celled embryos.

The newly fertilized egg contains two nucleoli, but this condition persists for only a very short time, since only one nucleolus is present after the first division of the fusion polar.

The synergids begin to degenerate soon after anthesis and before the pollen tube has penetrated into the cavity of the ovary. The first indication of a change is a slight yellow discoloration at the base of the synergids, followed by a disappearance of the large vacuole below the nucleus and a general telescoping of the cell, which takes on the shape illustrated in figure 2, *B*. The advance of the pollen tube to the proximity of the egg usually causes the destruction of one



A, Embryo sac with fertilized egg and dividing fusion polar. $\times 675$. B, Embryo sac with dividing fusion polar just above the antipodals. $\times 675$. C, Pollen tubes, the lower one containing two sperms. $\times 787$. D, Mature fertilized egg. $\times 1,380$. E, Pollen tube entering micropyle. $\times 397$.



A, Fertilized egg $\times 824$. *B*, Fertilized egg with two X-bodies $\times 600$. *C*, Fertilized egg with two X-bodies $\times 900$. *D*, Mitosis in fertilized egg. $\times 800$. *E*, Telophase in first division of fertilized egg. $\times 824$. *F*, 2-celled embryo $\times 664$. *G*, 4-celled embryo $\times 960$. *H*, 4-celled embryo with basal cell dividing $\times 1,120$. *I*, 4-celled embryo with two lower cells dividing simultaneously. $\times 960$

of the synergids, the second disappearing before the egg divides to form the embryo. In case of delayed pollination or fertilization the synergids remain unchanged for several days, but if an embryo fails to develop, they will degenerate prior to any other nuclei in the embryo sac.

THE TIME FACTOR

Since stained preparations of material fixed in Carnoy's solution show pollen tubes indistinctly, several hundred flowers were killed in a modification of Nawaschin's solution. The pollen tubes thus fixed stood out rather sharply and could be easily located in the cavity of the ovary and the micropylar canal.

Material fixed 22 hours after anthesis showed pollen tubes in the ovary cavity in all but one flower. In approximately 50 percent of the cases fertilization of the egg had already occurred. The fusion polar was in the caecum just below the antipodals and evidently not yet fertilized. Material fixed 24 hours after anthesis showed that all eggs were fertilized and each contained two nucleoli. The fusion polar was binucleolated in some flowers; in others there were already present two endosperm nuclei. Material fixed 28 hours after anthesis showed all flowers fertilized, but the eggs contained, at this stage, only one nucleolus. There were, without exception, two endosperm nuclei in each embryo sac.

It appears from this study that fertilization takes place approximately 1 day after anthesis; and since pollen germinates as early as 2 hours after the anthers dehisce, the time factor in fertilization is theoretically about 20 hours. However, in computing this factor it must be remembered that although fresh pollen may germinate within 2 hours after shedding, older and dried-off pollen germinates even sooner, and when it is applied to open instead of closed stigmas the time is likely to be still further reduced. Both fresh and dried-off pollen may germinate on unopened stigmas, but the growth on the unopened stigmas is slow, enabling the tubes from pollen applied several hours later to partly open stigmas to reach the embryo sac not far behind, perhaps even in advance of, those not so advantageously placed. This fact might have an important bearing on the question of cross-pollination, since the first germinating pollen belongs most likely to the same flower, while in free-blooming beets hybridization is the rule.

While this series of fertilization studies is based on material collected between June 19 and June 23, 1932, preparations from the previous season showed fertilization stages as early as 6 hours after anthesis. The data were, on the whole, not so uniform as those obtained in 1932, and showed much individual variation. However, the fact that in some beets numerous fertilization stages occurred as early as 10 hours after anthesis cannot be considered an accident, but rather argues that pollen-tube growth proceeds more rapidly in the flowers of some plants than in those of others. Oksijuk (6) observed fertilization as early as $3\frac{1}{2}$ hours after the flower opened, while 7 hours after anthesis about two thirds of them were fertilized. Since he does not describe his methods of marking the flowers, it appears, in view of the writers' data, rather doubtful whether fertilization normally takes place in so short a time after the pollen is shed.

Favorsky (3) tells of finding young embryos in tightly closed buds; but since fertilization of such flowers seems to be out of the question, he explains their presence as due to nucellar budding, a phenomenon that he had occasion to observe a number of times.

Weather conditions may and probably do have an influence on the time factor in fertilization. The month of June was warmer in 1931 than in 1932, and likewise the time required for the pollen tube to reach the embryo sac was shorter in the warmer month.

POST-FERTILIZATION PERIOD

The egg, unlike the primary endosperm nucleus, does not divide immediately after fertilization but undergoes a rest period, the length of which probably depends on both external factors and the genetic constitution of the plant. During this rest period general growth processes in ovule and embryo sac continue, while in the nuclei of the latter both progressive and retrogressive differentiations take place.

Measurements showing the progressive increase in the size of the ovule are given in table 2. The ovule at the time of anthesis has a long horizontal diameter of approximately 0.5 mm. It enlarges rapidly, having increased its linear dimensions approximately 40 percent at the time of fertilization (compare pl. 1, A, and pl. 3, A), and is almost fully grown, while the embryo is still very small. This rapid growth of the ovule during the early post-fertilization period is in harmony with the organization of the seed, which has a perisperm as a storage tissue. The endosperm has only a temporary existence and is consumed by the developing embryo.

Concomitant with the enlargement of the ovule is an increase in size of the embryo sac. The latter not only gains in length but broadens as well, especially during the first days after fertilization.

TABLE 2.— *Increase in size of ovule and embryo sac after anthesis*

Time after anthesis	Flowers examined	Longest horizontal diameter of ovule	Length of embryo sac	Width of embryo sac
	<i>Number</i>	μ	μ	μ
Check ^a	9	549	76	44
2 to 3 hours	63	569	82	48
4 to 5 hours	59	686	84	44
6 to 10 hours	27	686	80	43
17 to 20 hours	25	739	100	52
1 day	63	762	92	48
2 days	41	893	128	64
3 days	20	1, 258	172	104
4 days	21	1, 320	196	—
5 days	15	1, 918	—	—
6 days	6	2, 024	—	—
10 days	3	2, 464	—	—
Mature seed	4	2, 485	—	—

^a Examined at time of anthesis.

More conspicuous, however, than the enlargement of the embryo sac is the growth of the caecum, which is more or less an extension of the former below the antipodals. At the time of anthesis it is slightly shorter than the embryo sac but rapidly enlarges into a horseshoe-shaped cavity. This cavity is quite narrow but broadens somewhat at its distal end, which is easily recognized by an accumulation of

cytoplasm and free endosperm nuclei. The cells of the central nucellar tissue, which upon disintegration give rise to the caecum, are narrow and more elongated than the neighboring ones, and, according to Favorsky (3), they stain specifically with Pianese some time prior to disintegration. Soon after anthesis there appear in this specialized nucellar region narrow cavities which, as tissue breakdown spreads, unite into one common opening. This mode of formation is at times replaced by progressive tissue breakdown which has its beginning at the base of the embryo sac. In either case, however, the caecum is fully developed when the egg divides to form the embryo.

Both synergids and antipodals degenerate and disappear after fertilization. The synergids begin to degenerate soon after anthesis and have changed noticeably in appearance even before the pollen tube reaches the embryo sac. The pollen tube usually causes the

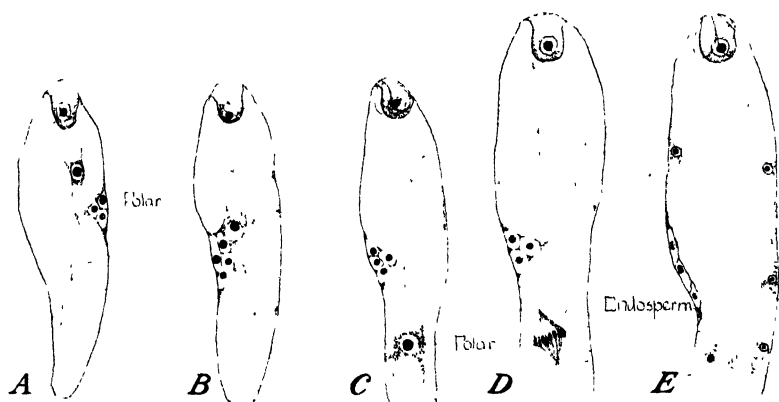


FIGURE 4. Diagrammatic drawings illustrating three characteristic positions of the fusion polar in the embryo sac: A, Polar above antipodals, B, polar opposite antipodals, C, polar below antipodals in the caecum, D, first division of fusion polar, E, marginal endosperm nuclei, the egg about to divide. (Compare with table 3.)

destruction of one of the synergids, and the complete degeneration of the other occurs soon afterwards.

The life of the antipodals, however, does not terminate with the advent of fertilization. There is usually an increase in their number, and the nuclei retain a normal structure for a number of days after fertilization, but finally, with the progressive destruction of nucellar tissue and the widening of the embryo sac, they become at first flattened and then degenerate and disappear.

The fusion polar or primary endosperm nucleus divides immediately after fertilization. Its position varies in the embryo sac (fig. 4). In young closed flowers, it is located midway between egg and antipodals, but as the flowers open, the polar usually moves to the lower end of the sac and thence into the caecum. A study of table 3, which gives the percentage distribution of the polar in relation to the antipodals, shows the fusion polar in the caecum below the antipodals in the majority of cases. Its position in the embryo sac, at a given age of the flower, does not appear to be bound up with the genetic constitution of the plant, although occasionally individuals will be found in which the fusion polar occupies one and the same

position in all the flowers of the same age. At the time of fertilization the fusion polar is normally in the caecum. Occasionally it is found near the antipodals or even above them, but whatever its position at this time, the daughter nuclei resulting from the first division will separate, one moving farther down in the caecum and the other in the opposite direction toward the egg. The first division of the polar is followed by a second and a third division, so that at the time the egg ends its rest period there will be present in the embryo sac a number of endosperm nuclei.

TABLE 3.—Position of fusion polar relative to the antipodals, mitosis in fusion polar and distribution of endosperm nuclei

Time after anthesis	Number of flowers examined	Percentage distribution of fusion polar relative to the antipodals, mitosis in fusion polar, and appearance of endosperm nuclei ^a				
		A	B	C	D	E
2 to 7 hours	376	18	11 9	64		
10 to 20 hours	75	10 6	9 7	40	4	36
24 hours	334	6 2	8 3	31 7	4 3	51 1

^a Letters correspond to those in figure 4.

EMBRYO DEVELOPMENT

FORMATION

After the fertilized egg has undergone a rest period it divides horizontally (pl. 5, *D*, *E*). Of the two daughter cells thus formed (pl. 5, *F*, and fig. 5, *A*), the lower one soon divides again transversely (fig. 5, *B*), and this is followed by a division of the upper or apical cell (fig. 5, *C*). These 2 divisions result in a tetrad of superimposed cells (pl. 5, *G*), of which the upper 3 form, by subsequent divisions, the embryo proper, while the basal cell gives rise to the hypophysis and the suspensor. Oksijuk (6) believes that the basal cell of the 2-celled embryo does not take part in the formation of the embryo, but that it enlarges and assumes the character of a haustorial cell. A study of plate 5 and figure 6, which give the sequence of cell divisions in the fertilized egg, fails to substantiate his contentions, and hence his criticism of the mode of embryo formation as given in an earlier publication by the senior writer (2) is, in the light of these new investigations, entirely unjustified.

Each element of the tetrad divides once to form the 8-celled embryo, but whereas in the formation of the tetrad a sequence pattern was rigidly adhered to, the time and place factors do not play such a vital role in the formation of the octant. The 4 cells of the tetrad are for convenience designated *a*, *b*, *c*, and *d*; *a* representing the apical and *d* the basal cell. Cell *d* usually divides first (pl. 5, *H*, and fig. 5, *D*), whereby the direction of the division wall is always transverse. Cell *c* of the tetrad divides later but sometimes simultaneously with *d*. The direction of the dividing wall may be either transverse or vertical (pl. 5, *I*, and fig. 5, *E*, *F*). Cells *a* and *b* of the tetrad may divide simultaneously (fig. 7, *B*), or, as is more often the

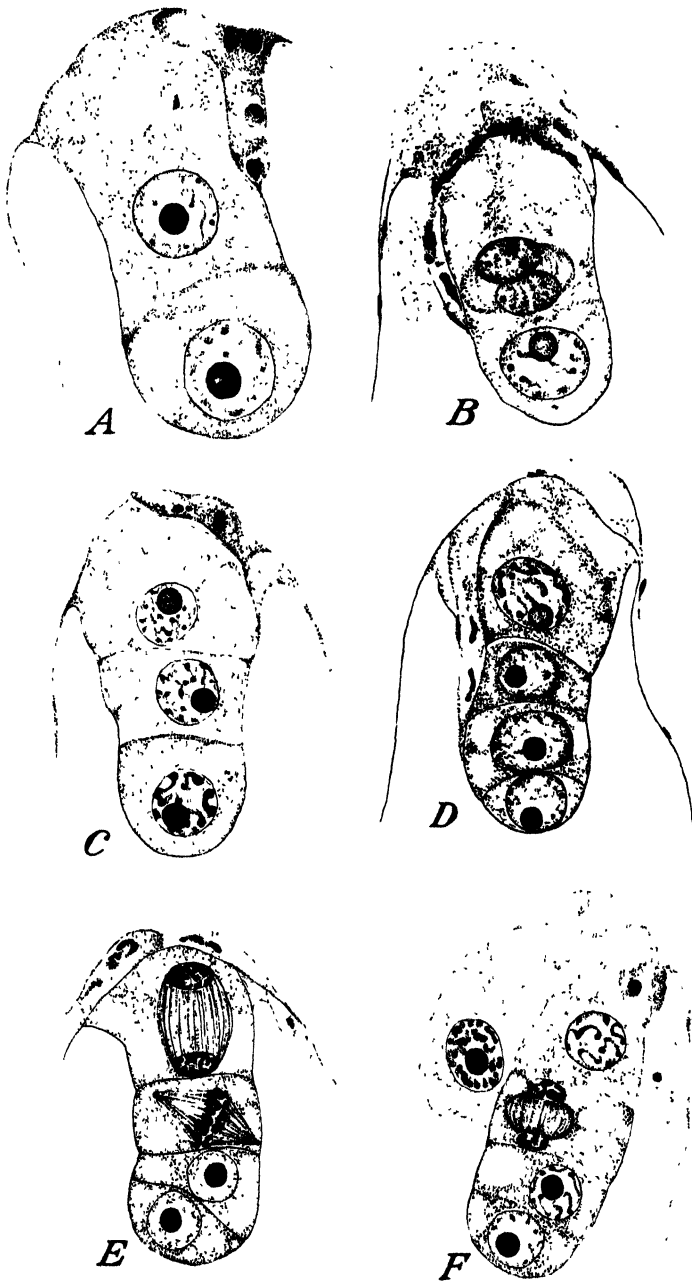


FIGURE 5.—A, 2-celled embryo with X-bodies still visible at the periphery of the basal cell, $\times 1,380$; B, 2-celled embryo with basal cell dividing, $\times 1,050$; C, 3-celled embryo with upper cell dividing, $\times 1,050$; D, 4-celled embryo with basal cell dividing, $\times 1,050$; E, 4-celled embryo with the 2 lower cells dividing, the spindle of the penultimate cell vertical to the long axis of the embryo, $\times 772$; F, same as E, but penultimate cell divides transversely instead of by a vertical wall, $\times 772$.

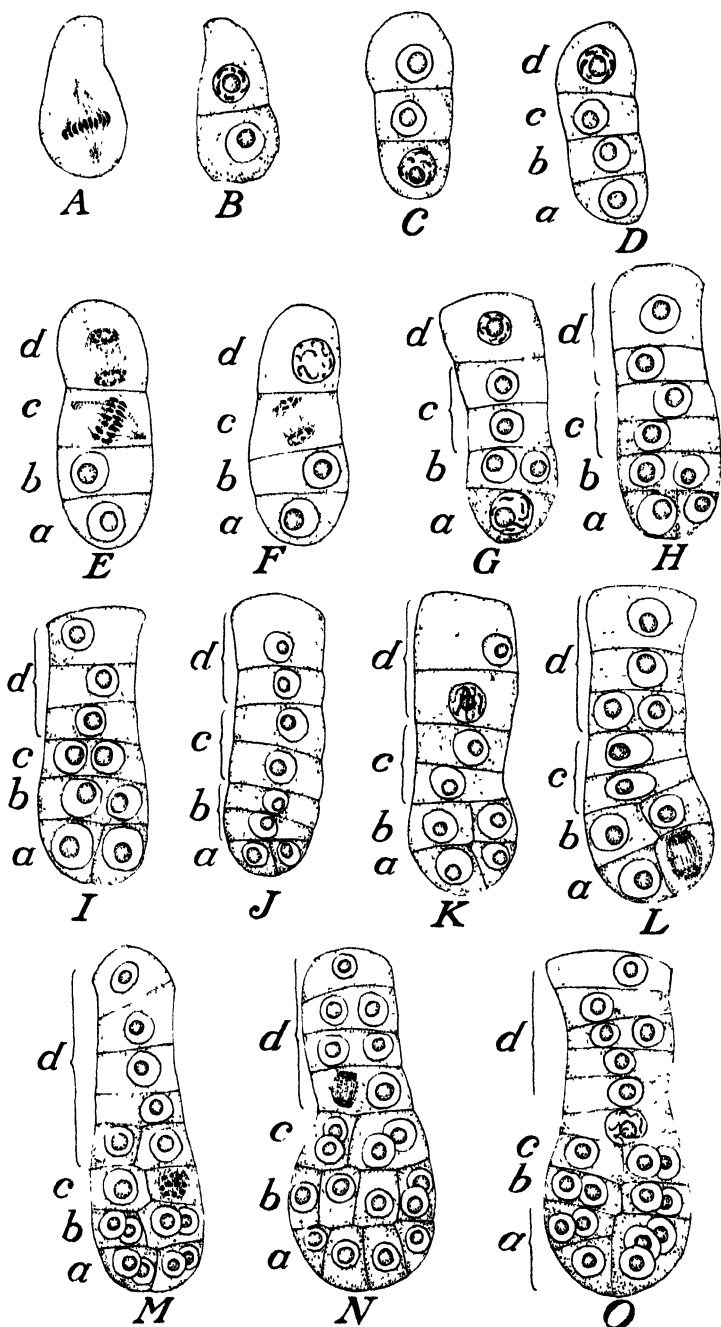


FIGURE 6.—A series of 15 diagrammatic drawings showing the sequence of cell division in the formation of the young embryo.



A, Similar to figure 7, *A*. $\times 850$. *B*, Similar to figure 7, *D*. $\times 722$. *C*, A 7-tiered octant. $\times 722$. *D*, Simultaneous mitosis in cells of a 6-tiered octant (for detail see figure 8, *D*). $\times 731$. *E*, Similar to *D*, except that cells are not dividing. $\times 731$. *F*, 16-celled embryo. $\times 595$. *G*, Octant with penultimate cell dividing. $\times 867$. *H*, Globular embryo surrounded by cellular endosperm. $\times 212$.

case, one may divide before the other (fig. 7, *A*, and pl. 6, *A*). Cell *a* always divides by a vertical wall, but *b* may divide either transversely (fig. 7, *C*, *E*) or vertically (pl. 6, *A*, *B*, *E*, *F*, and *G*, and fig. 7, *A*, *D*, *F*), the latter mode of division being the more frequent. Thus, depending on the direction of the division walls in *b* and *c*, the 8-celled embryo is either 5-, 6-, or 7-tiered (pl. 6, *C*). The formation of the tetrad in the sugar beet tallies with the development of the pro-embryo in *Chenopodium*, as described by Souèges (8), but the subsequent divisions in the latter differ in that cell *B* always and cell *c* usually divide by vertical walls so that the octant is commonly 5-tiered.

The 16-celled embryo (fig. 8, *D*) is formed by the division of the elements of the 8-celled embryo. The direction of the division walls will largely be governed by the behavior of the cells of the tetrad. If the resulting octant is 5-tiered, the 2 superimposed daughter cells of *d* divide again horizontally to form a row of 4 cells. Cell *c*, which divided by a vertical wall in the formation of the octant, now divides transversely, while the daughter cells of *a* and *b* divide again vertically. The resulting 16-celled embryo has 8 tiers with the upper 2 forming in cross section each a quadrant. If either *b* or *c* of the 4-celled embryo divided transversely the resulting octant would be 6-tiered (pl. 6, *D*, and fig. 8, *A*, *C*), and in order for the 16-celled embryo to have the normal number of 8 tiers the division walls of the 6-tiered octant would differ somewhat from those of the 5-tiered octant. If the first division of *c* were transverse (fig. 8, *B*) the second division would be vertical, whereby the normal tiered arrangement of the 16-celled embryo would be established. In case *b* divided transversely in the formation of the octant, equalization in the normal number of tiers would not be attained in the embryo until after a series of subsequent divisions. The same holds true for the 7-tiered octant. An examination of figure 6 shows other irregularities in the sequence of cell division which give the impression that, after the formation of the quadrant, no strict law governs the sequence of cell division. However, a certain balance seems to be maintained so that the final product is remarkably uniform.

THE TIME FACTOR

Two-celled embryos have been observed as early as 24 hours after anthesis, but in the majority of cases the egg does not divide until 36 hours after the flower has opened. It has previously been shown that fertilization is effected within a day after anthesis or even sooner, so that the rest period of the egg equals the time necessary for the pollen tube to reach the embryo sac. This view, substantiated by a large number of observations on carefully marked material, must needs take the place of an earlier opinion of the senior writer that the fertilized egg divided immediately or after a short period of rest. There must likewise be a revision in the views of other writers, who, although correctly assuming a rest period, find 2-celled embryos about 4 days after anthesis, whereas normally most eggs have already divided 2 days after the flowers open.

The time factor in the early embryogeny of the sugar beet is illustrated in table 4. It will be seen that early growth and differentiation are extremely slow. Eight-celled embryos are most plentiful

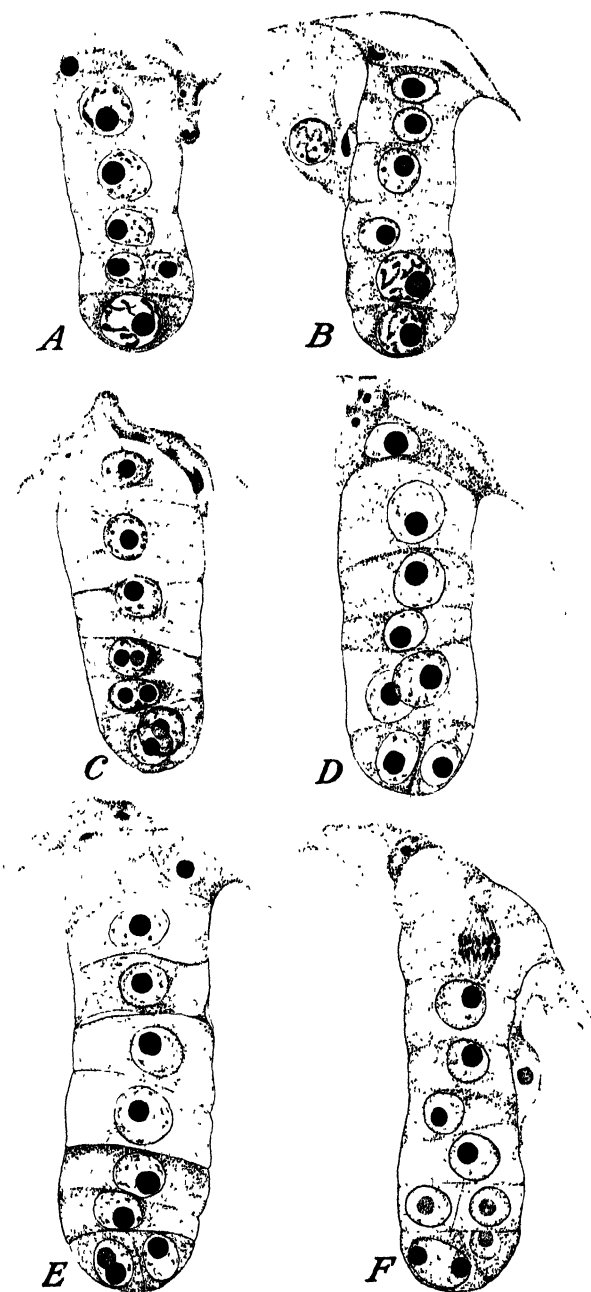


FIGURE 7—A, 5-tiered embryo with the cell next to the apical divided in advance of the apical cell, $\times 1,260$; B, 6-tiered embryo showing simultaneous division in the 2 apical cells; C, a later stage; while the apical cell is dividing vertically, the cell next to it has divided horizontally, $\times 1,260$; D, similar to C except that the cell next to the apical is dividing by a vertical wall, $\times 1,260$; E, a later stage of C, $\times 1,260$; F, a stage differing from the preceding one principally in that the cell next to the apical has divided vertically, $\times 1,260$.

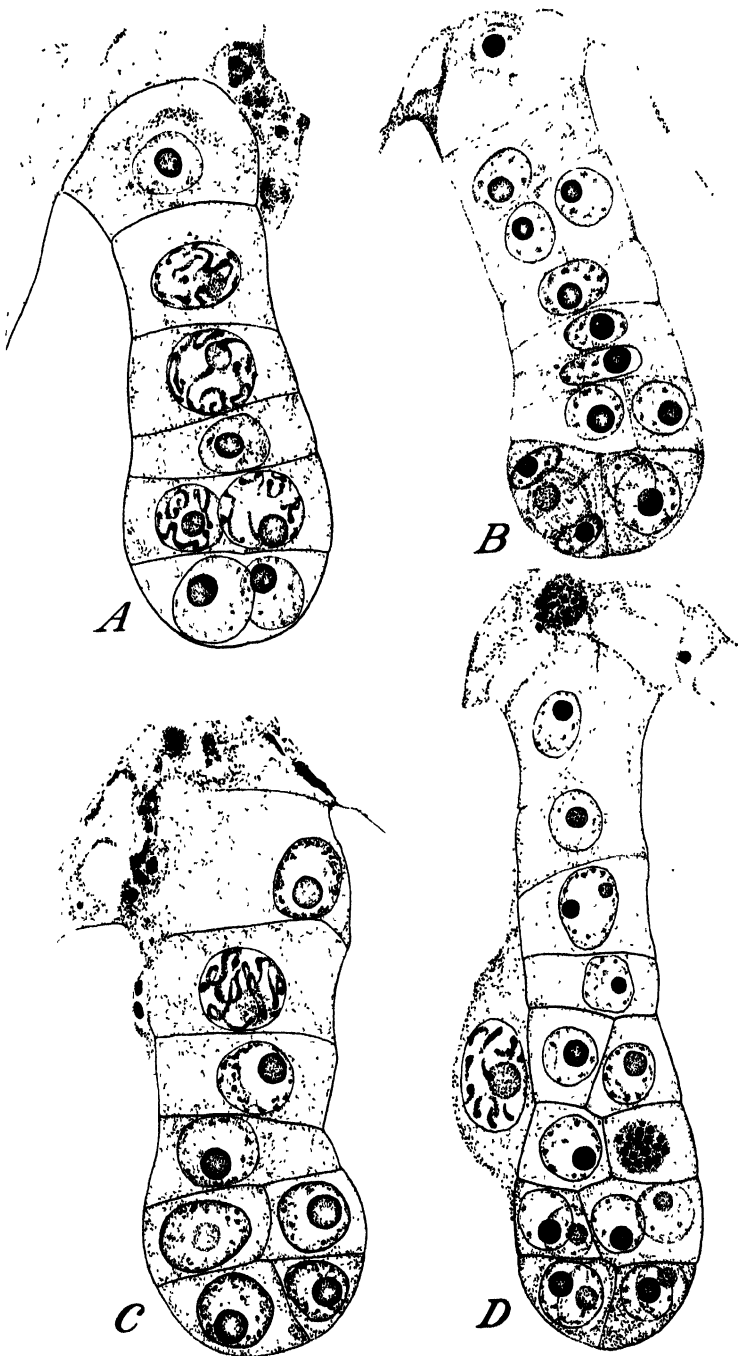


FIGURE 8.—A, Detailed drawing of plate 6, D, $\times 1,530$; B, cell division in the octant, $\times 1,190$; C, detailed drawing of plate 6, G, $\times 1,530$; D, 16-celled embryo, with a laterally attached endosperm nucleus, $\times 1,190$.

from 2½ to 3 days after anthesis, while club-shaped embryos of 16 cells or more dominate the picture about 1 day later. The globular form (pl. 6, II) appears about the fifth day, and differentiation of the cotyledons a day or two later. Growth continues very slowly up to the eighth or ninth day, and thence rapidly, so that the embryo is mature by the twelfth or thirteenth day.

TABLE 4.—*The time factor in embryo development*

Time after anthesis (days)	Flowers exam- ined	Ferti- lized eggs	Eggs dividing	2- to 4- celled embryo	4- to 5- celled embryo	6- to 8- celled embryo	16-celled embryo	Older embryo
	Number	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1	334	86.5		9.7	2	1.4		
2	137	27	8.7	24	22.5	15.3	2.1	
2½	53	9.4		7.4	24.4	90.2		
4	61				3.1	11.3	8.1	77

Concomitant with the differentiation of the embryo is the development and subsequent disappearance of the endosperm. Its development is initiated with the fertilization of the fusion polar, which, unlike the egg, does not pass through a rest period. The endosperm nuclei migrate to the periphery of the sac, where they become embedded in cytoplasm. At the termination of the rest period of the egg there are present in the embryo sac from 4 to 6 nuclei and not infrequently an even larger number. Mitosis in the endosperm nuclei is simultaneous. The period of free nuclear division continues to the fifth day, when the embryo has attained a globular form. The nuclei then become invested by walls, and gradually the entire cavity of the embryo sac becomes filled with a delicate tissue, which, however, is again dissolved and the contents absorbed by the growing embryo.

SUMMARY

This investigation is based on a study of about 3,000 sugar-beet flowers collected during the month of June 1931 and 1932 at Rocky Ford, Colo. The flowers were marked at the time of anthesis, and collections were made at definite intervals.

The stigma of the young beet flower is closed. It begins to open in midafternoon, approximately 7 hours after anthesis, and expansion is completed within 24 to 36 hours; occasionally the stigma begins to open sooner and the lobes are fully expanded a few hours later.

The embryo sac has the normal nuclear complex. The fusion polar migrates into the caecum sometimes even before anthesis. The caecum begins to form before the flower opens, and its development is completed soon after the egg is fertilized.

The ovary with its ovule and embryo sac has usually a normal structure. There have been observed the following abnormalities: 2 ovules within an ovary; 2 eggs within an embryo sac; abnormal elongation of nucellar tip and inner integument; partial or complete suppression of the embryo sac.

The pollen of a given flower is fairly uniform in size, since grains of extreme dimensions are relatively few. Pollen grains of different selections have a different average diameter, but their plotted measurements rarely give a bimodal curve as claimed by Oksijuk (6) for

his material. A great deal of pollen degenerates, but, even so, many pollen grains germinate and grow down the stylar canal into the cavity of the ovary, and as a rule more than one pollen tube reaches the embryo sac. The time necessary for the pollen tube to reach its destination is about 20 hours. In some flowers this time factor is reduced to 10 hours or even less.

Fertilization is accomplished soon after the pollen tube reaches the embryo sac. Accompanying this process are certain dark-staining bodies which usually flank the egg in pairs and constitute in most cases supernumerary sperms.

The synergids begin to degenerate before fertilization, but the antipodals very often increase in number and do not disappear until embryo development is well under way.

The fusion polar divides without undergoing a rest period. At the time of fertilization there are usually present from 4 to 6 endosperm nuclei.

The egg undergoes a rest period which approximately equals the time it takes the pollen tube to reach the micropyle. Two-celled embryos are found commonly 36 hours after anthesis and occasionally even sooner. In the subsequent development of the embryo, growth is at first very slow. Differentiation of the cotyledons is noticeable in about 6 days, or somewhat later than the appearance of cellular endosperm, and the embryo is mature from 12 to 14 days after anthesis. The first divisions of the egg are transverse and result in a tetrad of superimposed cells, of which the upper three form the embryo proper, while the basal cell gives rise to the hypophysis and the suspensor.

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CYTOLOGICAL FEATURES OF NICOTIANA GLUTINOSA HAPLONTS¹

By JOHN MILTON WEBBER²

Associate cytologist, Division of Egyptian Cotton Breeding, Bureau of Plant Industry, United States Department of Agriculture

This paper represents the sixth in a series, the first five numbers of which appeared in the University of California Publications in Botany (1, 14, 22, 23, 24).³ The investigation on which this paper is based was begun while the writer was a research associate in the department of botany at the University of California, and was completed after he joined the staff of the Bureau of Plant Industry, United States Department of Agriculture.

INTRODUCTION

Nicotiana glutinosa L. has been grown in pure line for over 25 years in the botanical gardens of the University of California. It is one of the most distinct species of the genus and is strictly monomorphic. The leaves are small, cordate, long-petioled, and extremely glandular. The flowers, which are light yellow tinged with deep red, are short-cylindrical at the base, but above the base they expand abruptly into an irregular, obliquely one-sided funnel. The limb is somewhat bilabiate. The stigma and anthers are connivent just under the middle lobe of the upper lip. Although the species is included in the *N. rustica* L. section of the genus (?) its zygomorphic flowers show a close relationship to *N. tomentosa* Ruiz and Pav. of the *N. tabacum* L. section. It possesses 12 pairs of chromosomes.

Three *Nicotiana glutinosa* haplonts have been found among the plants in the botanical garden. Although the first of these was discovered among 24 seedlings that were subjected to X-radiation, the variation was apparently spontaneous and not the result of the treatment (11). The two other haplonts, 30.179-9 and 30.180-49, occurred in two F₃ populations of 50 plants each, grown from X-rayed seed during the spring of 1930. The parents of both of these populations were selected as having lower vigor and fertility. It is probable that these two haplonts were due to haploid parthenogenesis stimulated by an increased incompatibility of male and female gametes.

Except for considerable reduction in all the parts, the haplonts are normal in appearance. The flowers are slightly lighter in color, and the anthers and stigma, although noticeably smaller, are essentially normal. The plants are self-sterile and produce no seed when pollinated with diploint pollen. Photographs of diploid and haploid plants are shown in figures 1 and 2.

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² The writer is much indebted to Dr. T. H. Goodspeed, professor of botany, University of California, for the experimental material and for the opportunity to pursue the study, and to Dr. I. E. Webber, of the Division of Blister Rust Control, Bureau of Plant Industry, for helpful suggestions in regard to histology and for aid in the preparation of the manuscript.

³ Reference is made by number (*italic*) to Literature Cited, p. 866.

Haplonts in general exhibit many interesting cytological phenomena, some of which may serve as a basis for the interpretation of certain complex processes of meiosis in diplonts and hybrid plants. The early prophase stages of haplonts furnish evidence regarding the origin and nature of the double chromosomes seen in the later stages of the



FIGURE 1.—*Nicotiana glutinosa*: A, Diplont 30.180-48; B, haplont 30.180-49.

diplont. Since, in general, haplonts form univalent chromosomes in the first metaphase, many irregularities occur during the remaining stages of sporogenesis. Such irregularities as univalent division, fragmentation, and elimination are frequent and exceptionally clear. The formation and behavior of binucleate germ cells and of unreduced gametes and finally the steps of degeneration are likewise frequent and

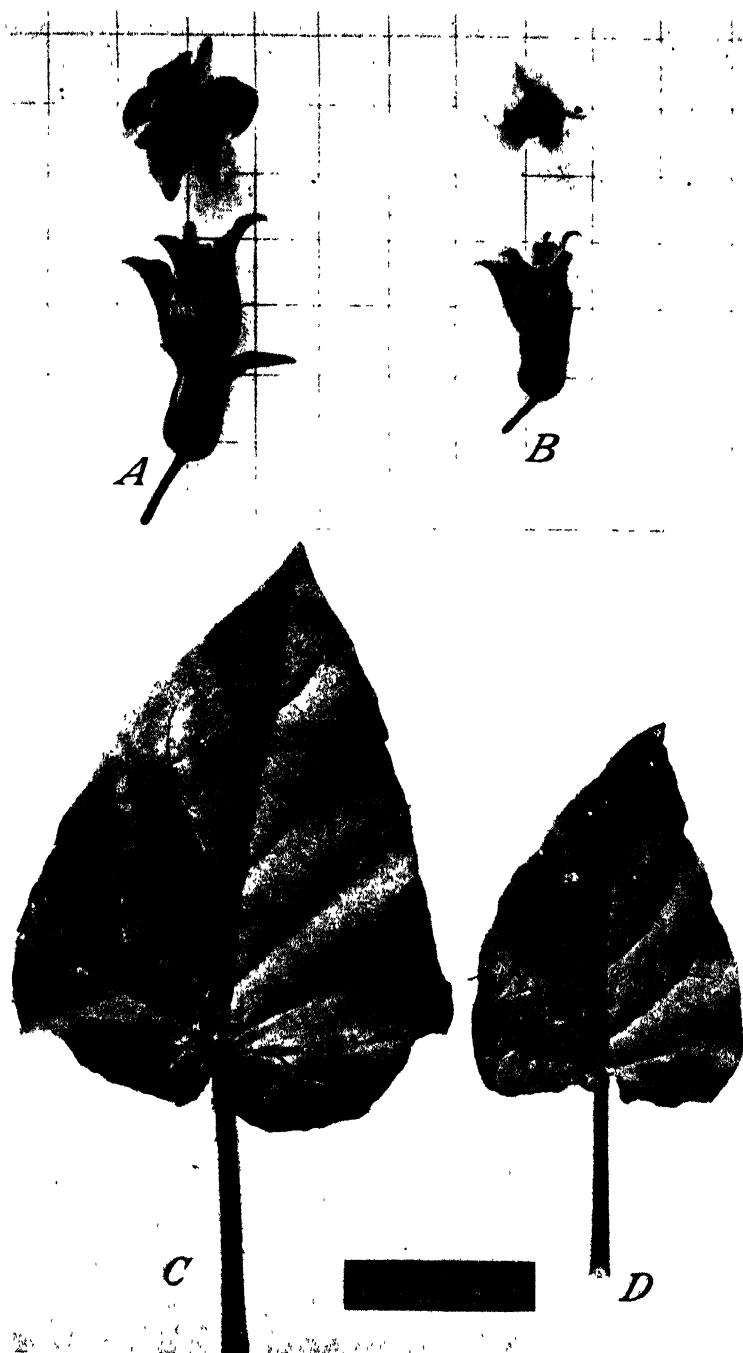


FIGURE 2.—*Nicotiana glutinosa*: A, Typical flowers of diplont 30.180-48, shown against background ruled in centimeters; B, typical flowers of haplont 30.180-49; C, typical leaf of diplont 30.180-48; D, typical leaf of haplont 30.180-49.

clear. Similar products of such irregularities observed in other plants doubtless originated in many cases from phenomena closely related to those occurring in haplonts.

Because of the reduced number of chromosomes in the somatic cells, the root tips are obviously most favorable for the study of chromosome morphology. Moreover, such root tips frequently contain diploid cells and tissues. The formation of diploid areas in haplont roots and comparisons of such areas with tetraploid areas in roots of diplonts are extremely interesting. The cytology of root initials, callus tissues, and cuttings presents evidence as to the nature of diploid areas in haplonts and their relative frequency of occurrence in root and shoot.

Studies in chromosome number and morphology in *Nicotiana* have demonstrated that characteristic chromosome features distinguish certain species. The results for *N. tabacum* (23), *N. alata* Link and Otto (1, 22), *N. longiflora* Cav. (14), *N. sylvestris* Spegaz. (24), and *N. langsdorffii* Schrank,⁴ together with the data included herein, indicate that specific chromosome features furnish evidence as to the origin and relationship of species within the genus. It is encouraging that such evidence is accumulating and that it harmonizes closely with the evidence obtained from interspecific hybridization.

MATERIAL AND METHODS

The seeds giving rise to *Nicotiana glutinosa* haplonts 30.179-9 and 30.180-49 and to sister diplonts were germinated in pots. The seedlings were later transplanted into flats; when approximately 6 weeks old, they were set out in the field.

When approximately 16 weeks old, plants 30.179-9 and 30.180-49 were suspected of being haplonts and a few root tips were carefully collected from each plant. Soon after this 30.179-9 was injured and died. When 22 weeks old, 30.180-49 was known to be a haplont, and anther, ovary, and additional root-tip material were collected. At this time the plant was potted, removed to the greenhouse, and 20 stem cuttings were made.

Two weeks later root tips were gathered at random and from marked roots of the haplont. When the plant was 26 weeks old, root tips were similarly collected, and material for a study of root initials was obtained. By this time the roots had become so pot-bound that it was necessary to remove all exposed roots. Subsequent root-tip collections were made when the plant was 28, 30, 35, 37, and 41 weeks old. All exposed roots were removed after the root-tip collection made when the plant was 37 weeks old.

During this period and until the writer last saw the haplont, when it was 1½ years of age, it continued to grow vigorously and to produce numerous flowers. Frequent cytological examinations showed that its shoots remained haploid. As previously stated, the sexual cells of the plant were highly sterile.

Of the 20 cuttings, 13 produced callus tissues over their proximal ends and 7 died. Two of the surviving 13 were used for the study of callus tissue and root initials. In order to stimulate distal callus formation, a fresh cut was made across the apical internode of 5 of the

⁴ RAUP, L. J. MEIOTIC BEHAVIOR OF NICOTIANA GLUTINOSA HAPLOIDS. (Thesis, University of California.) [Unpublished.]

remaining cuttings. The cuts on 3 of these were immediately covered with petrolatum. All 5 were kept in a humid chamber and only 1 shoot bearing 2 leaves was allowed to develop on each. In no case did the cuttings form callus tissue at the distal end. The remaining 6 cuttings were allowed to develop, and their root tips and flower buds were subjected to cytological examination. All shoots produced were haploid and remained haploid until the plants died.

Diploid material of *Nicotiana glutinosa* was obtained from plants 27.028-6, 27.028-7, 28.028-53, and 30.005-1. The populations containing these four plants were grown from pure untreated seed.

The cytological observations were made on paraffin material, permanent smears, and acetocarmine smears. All cut material was killed and fixed in the following chromoacetic-formalin mixture:

- 1 part: 65 cc water, 10 cc glacial acetic acid, and 1 g chromic acid.
- 1 part: 40 cc commercial formalin and 35 cc water.

Before paraffin infiltration, callus tissues were softened in diaphenol. Paraffin sections were cut 10μ thick and stained in Heidenhain's iron haematoxylin.

Somatic chromosome drawings and measurements were obtained from division figures located in the upper portion of the zone of cell division in the periblem. In this region the chromosomes vary only slightly in size and shape.

Camera-lucida drawings were corrected by direct observation. The drawings are reproduced at the following diameters: Somatic chromosomes and callus cells, at 4,750; pollen mother cells and sporads, at 2,000; and female gametophytes, at 950. No attempt has been made to show the relative levels at which the chromosomes lie.

CHROMOSOME MORPHOLOGY IN HAPLONT AND DIPLONT NICOTIANA GLUTINOSA

The haploid chromosome number of *Nicotiana glutinosa*, recorded by Goodspeed (9, 10) as 12, has been repeatedly confirmed by cytologists. Christoff (3) determined and figured the 24 somatic chromosomes of the species. His figures clearly show that the chromosomes of the complex vary but little in size. He found that the somatic chromosomes of *N. glutinosa* are shorter and slenderer than those of *N. paniculata*.

In the present study a large number of favorable somatic metaphase plates were examined in root tips from both haplonts and diplonts. A casual observation of diploid plates (pl. 1, G-I) gives the impression that the chromosomes are all of approximately the same size but readily divisible into a group of V-shaped and a group of rod-shaped chromosomes. However, a detailed study of these chromosomes shows slight variations in size and form within each of the two groups.

A close examination of the chromosomes composing the V-shaped group shows that they may be classified under six types (fig. 3). Type 1 comprises two pairs of chromosomes having median constrictions. Each segment of this group of four chromosomes averages 1.80μ in length. Type 2 likewise comprises two pairs of medianly constricted chromosomes. However, the segments of this type average 2.00μ in length. Type 3 comprises one pair of medianly constricted chromosomes, the segments of which average 2.26μ in length. Obviously the chromosomes of the foregoing five pairs are

strictly V-shaped and are very nearly the same in size, which makes it difficult to identify them in diploid plates.

Type 4 comprises two pairs of submedianly constricted chromosomes, each with segments averaging 1.60μ and 2.15μ in length. Type 5 comprises a single pair of submedianly constricted chromosomes. The segments of this pair average 1.80μ and 2.26μ in length. The chromosomes of types 4 and 5, as in the case of types 1, 2, and 3,

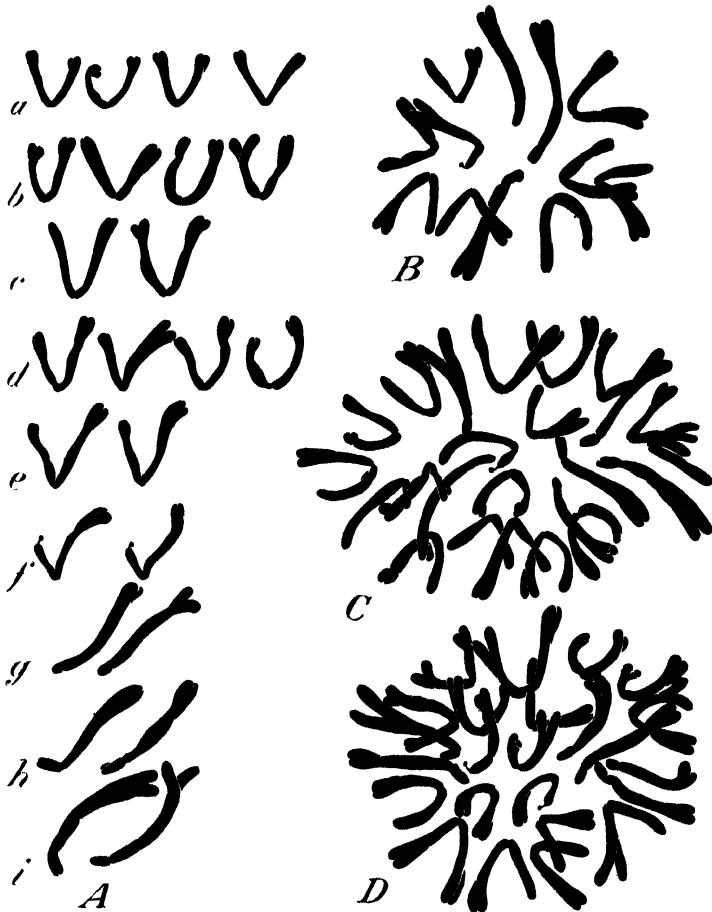
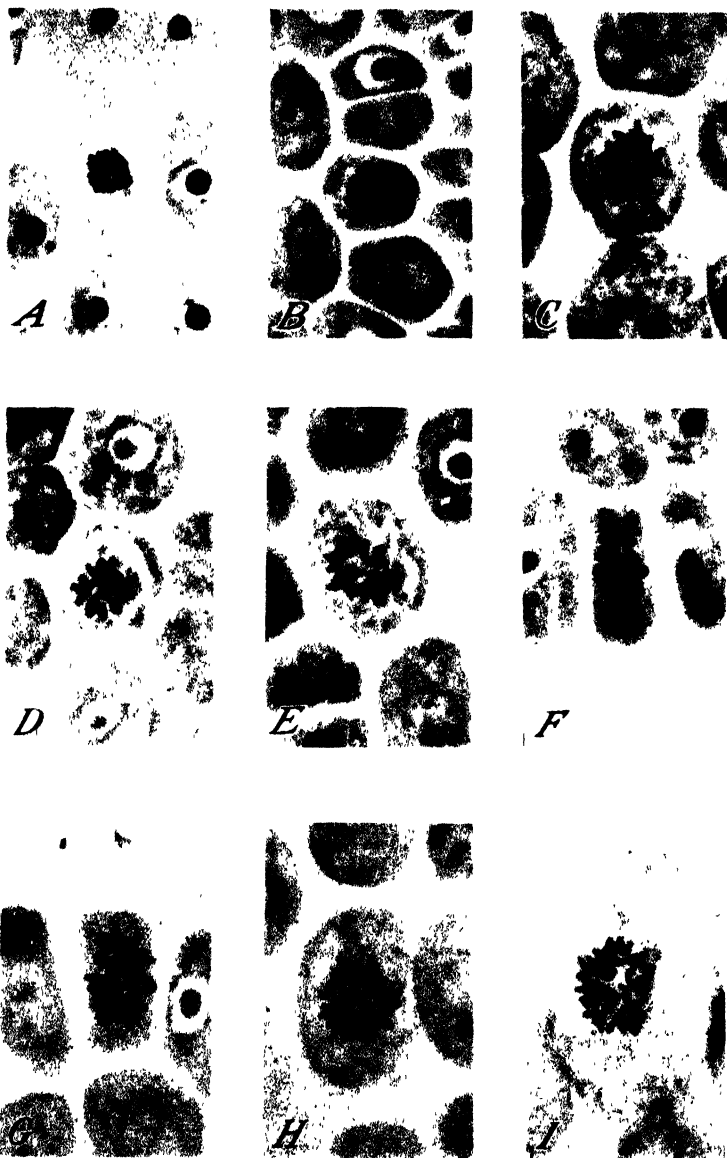


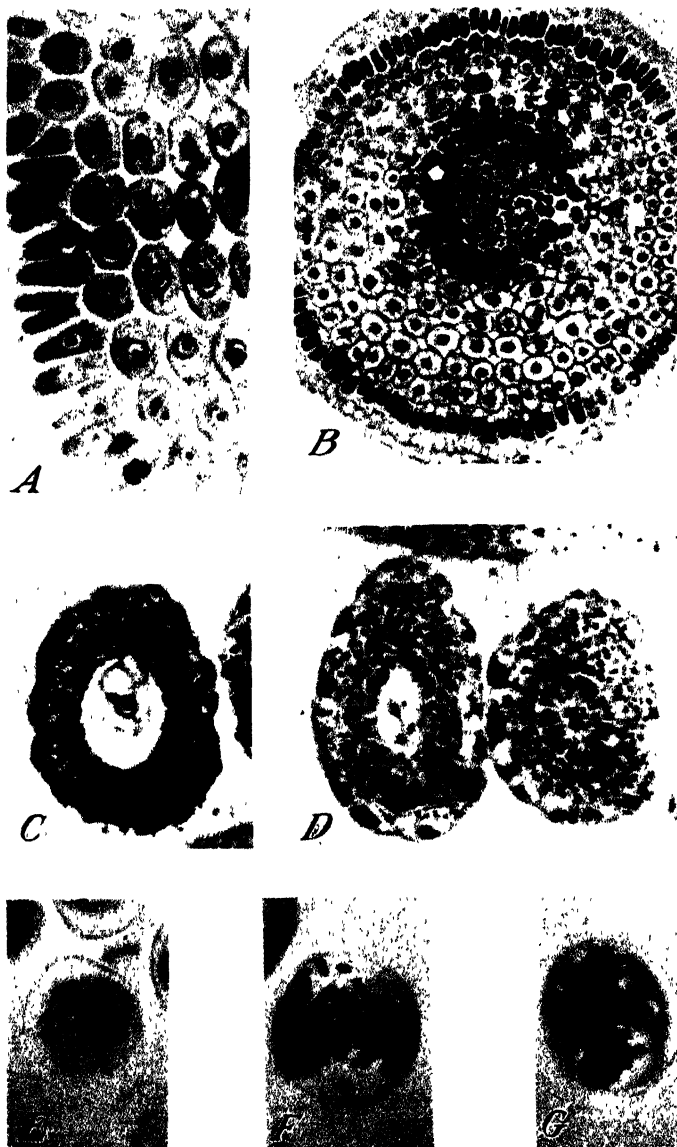
FIGURE 3.—Somatic chromosomes of *Nicotiana glutinosa*: A, The nine identified chromosome types; B, haploid plate from haplont 30.180-49, C, diploid plate from haplont 30.180-49; D, diploid plate from diploid 27.028-6. $\times 4,750$.

are very similar but are easily distinguishable from the chromosomes of the first three types because of their submedian constriction and consequent approach to an L-shape.

The pair of chromosomes composing type 6 is the most easily distinguished of the entire complex. The chromosomes of this type have submedian constrictions, are decidedly L-shaped, and have well-defined proximal satellites. The segments of the pair average 1.40μ and 2.40μ in length.



Nicotiana glutinosa.—Typical metaphase plates from root tips of haplont 30 180-49. A-F, Haploid plates. A and B, Constricted chromosomes. G and H, Diploid plates. I, Typical metaphase plate from root tip of diploont 27 028-6.



Nicotiana glutinosa haplont 30 180-49. A, Haploid root tip with a small diploid area in the dermatogen. B, Haploid root tip with a large diploid area in the perilem. This chimera nearly surrounds the pleome and greatly distorts the usual symmetrical cell arrangement of the tip. C, Embryo sac with a single large nucleus. Compare with D. D, At left, embryo sac with four characteristic nuclei; at right, characteristic disintegrated embryo sac. E, Pollen mother cell. Abnormal interkinesis. Daughter nuclei are connected by chromatin thread. Compare with F. F, Pollen mother cell. Interkinesis. Chromatin elements are well separated and clearly exhibit homotypic split. This condition is characteristic for the stage in both haplonts and diplonts. This mother cell is larger than that shown in E and contains more than 12 chromosome elements; it probably represents an advanced stage of a binucleate prophase (G). G, Pollen mother cell. Binucleate synizesis, showing characteristic chromatin granule in plasma, and well-separated nuclei.

Upon close examination it is found that the chromosomes of the rod-shaped group may likewise be classified as types (fig. 3). Type 7 comprises the smallest pair of chromosomes of the complex. The chromosomes of this pair average only 3.40μ in length and have practically terminal constrictions. Type 8 likewise comprises a single pair of chromosomes. The constrictions of this pair, however, are further removed from the proximal end than are those of the preceding pair, and the chromosomes are slightly longer, averaging 3.70μ . The chromosomes of the pair composing type 9 are similar to those of type 8 in that they have subterminal constrictions. These chromosomes, however, average 0.80μ longer than those of type 8. Although the chromosomes of the three latter types do not exhibit greater differences than those of types 1, 2, and 3 or types 4 and 5, they are more easily identified. The chromosomes of the rod-shaped group are less twisted and bent than are those of the V-shaped group. Hence, in the former group, differences in form and size are more striking and more easily measured.

A casual observation of haploid plates (pl. 1, A-F) clearly shows that there are 9 V-shaped and 3 rod-shaped chromosomes. A detailed study of such plates shows the presence of the 6 types of V-shaped and the 3 types of rod-shaped chromosomes described in connection with the diploid plates. Owing to the fewer number of chromosomes in such plates, the chromosome types are more easily identified than in the diploid plates.

The somatic chromosome types just described, in addition to being characteristic of the two haplonts 30.179-9 and 30.180-49 and all diplonts examined, are also distinguishable in the diploid cells in the root tips of haplonts (pl. 1 and fig. 3). It is likewise possible to distinguish 12 rod-shaped chromosomes and to identify 4 chromosomes of type 6 in one tetraploid metaphase in a diploid root tip from a haplont.

In root-tip metaphase plates of *Nicotiana sylvestris*, the writer (24) has noted that the chromosomes often appear to lie in pairs. A similar orientation is apparent in diploid metaphase plates of *N. glutinosa* (fig. 3, C). The writer (25) has suggested that such orientation is due to similarity of form or the location of spindle-fiber constrictions. This suggestion is supported by the fact that similar chromosomes in haploid *N. glutinosa* plates often appear to lie together or to occupy similar positions within the achromatic figure (pl. 1, F; fig. 3, B).

OCCURRENCE OF DIPLOID TISSUES IN HAPLONT NICOTIANA GLUTINOSA

Large portions of the root tips of haplont 30.180-49 were entirely or partly diploid (pl. 2, A and B). Diploid cells and the boundaries between diploid and haploid areas may be rather accurately determined on the basis of cell and nuclear size and chromosome number.

Table 1 shows the number of root tips examined and the approximate age of the plant when they were collected. In the case of each root tip, the data are based on a careful examination from the root cap to the few scattered divisions in the upper zone of cell enlargement.

TABLE 1.—*Frequency of diploidy in root tips of Nicotiana glutinosa haplont 30.180-49^a*

Age of haplont (weeks)	Number of root tips				Percentage of diploid tips
	Haplont	Diploid	Partly diploid	Total	
16	17			17	0
22	22			22	0
24	33	2		35	5.71
26	40	6	1	47	12.77
28	23	2	1	26	7.69
30	50	^b 17	1	68	25.00
35	20	9		29	31.03
37	8	10		18	55.56
41	41	30	3	74	40.54
Total	254	76	6	336	22.62

^a The condition of the haplont at times of root-tip collection is described under Material and Methods. All tips were collected at random.

^b 1 tip contained a small tetraploid area

From the data in table 1 it appears that the haplont formed few or no diploid root tips until it was more than 22 weeks old. After this period the percentage of diploid tips gradually increased until the thirty-seventh week, when 55.56 percent of the root tips examined were wholly diploid. As noted in a preceding section, the plant was removed from the field to the greenhouse at the end of 22 weeks, and after 26 and 37 weeks the roots were severely cut back. These facts may account for the sudden appearance of diploid roots at 24 weeks and the slight decrease in the percentage of diploid roots following the twenty-sixth and the thirty-seventh week.

Of the 6 haplont root tips containing $2n$ areas, 2 had diploid areas in the dermatogen (pl. 2, A), 2 in the plerome, and 2 in the periblem. Four of the diploid areas were comparatively small and limited to either the zone of cell division or the zone of cell enlargement. One, occurring in the plerome, extended from the zone of cell enlargement to the root cap. The remaining diploid area, occurring in the periblem, extended from the last section examined in the zone of cell enlargement into the root cap, or approximately 820μ . This diploid area (pl. 2, B) nearly surrounded the plerome and greatly distorted the usual symmetrical cell arrangement of the root tip. Each of the two last-mentioned diploid areas contained more cells and formed a greater proportion of the root tip in the zone of cell division than in the zone of cell enlargement.

The small tetraploid area in a diploid root tip was situated in the periblem of the upper portion of the zone of cell division. It was composed of from 8 to 10 cells, 1 being in the metaphase stage of mitosis.

Marked roots, which had produced either wholly haplont or wholly diploid tips, were found to continue to produce their respective type for a period of over 2 weeks. Fifteen root tips were examined from the marked haplont root and 19 from the marked diploid root.

Of 19 pieces of rootlets gathered at random from the haplont, 13 had wholly diploid root initials, 5 had wholly haplont initials, and 1 had 1 haplont and 2 diploid initials. There was a total of 32 diploid and 10 haplont initials. This material was collected at the end of 26

weeks. Hence, the high percentage of diploid initials indicates that if the roots had not been cut back at 26 weeks the percentage of $2n$ tips at 28 weeks (table 1) would have been considerably higher.

The callus covering the proximal or root end of the cuttings apparently originated from the vascular cambium. Unfortunately the cells of the forming callus were very irregular in shape and arrangement, so that the mitotic figures were difficult to study. The bulk of the callus, however, was composed of haploid cells. In the forming callus of 1 cutting 4 isolated diploid cells and 1 diploid area composed of approximately 5 cells were visible. In such callus tissues binucleate cells were about as common as diploid cells.

Although several root initials were visible in the callus, in no case did they arise within this tissue. Apparently the initials always originate from the vascular cambium located several sections above the callus. The young initials grow into the callus and finally through it. The callus thus forms a protective tissue for the initials. However, since the initials penetrate the callus more readily than the epidermis, callus tissue must afford less protection than epidermal tissue.

In 1 cutting 5 initials were visible in the callus and 1 in the cortex. In sectioning, the initials were cut at various angles and hence were difficult to study. However, all initials were apparently wholly haploid.

Of the 6 cuttings allowed to develop, only 1 formed diploid root tips in its early history. Two of the first 12 tips collected from this plant were diploid. The first diploid tips on the remaining 5 cuttings appeared approximately 3 weeks later than on the other plant. As in the case of the parent haplont, after the cuttings began to form diploid root tips, the percentage of such tips gradually increased. Of the 130 root tips examined from these cuttings, 22 were wholly diploid and 2 partly diploid.

The significance of the cytological evidence already submitted will be discussed later. It may be noted here that since the extent of diploidy is similar in root tips, marked roots, and root initials, the findings in each confirm those in the others. Moreover, the extent and nature of diploidy in haplont *Nicotiana glutinosa* is very similar to that found in haplonts of *N. tabacum* (23), *Crepis capillaris* (15), and *Lycopersicum esculentum* (19).

CHROMOSOME DOUBLING IN HAPLONT NICOTIANA GLUTINOSA

Several observations indicate that chromosome doubling in the root tips of haplont *Nicotiana glutinosa* occurs through failure of anaphase separation. In the majority of cases the stages of mitoses in haploid tips are as regular as those in diploid tips. Occasionally, however, in haploid figures, the nucleolus does not disappear during the prophase. Remnants or traces of the nucleolus are present during the metaphase and rarely during the anaphase. In the latter stages (fig. 4, A), the achromatic figure and the majority of chromosomes are apparently normal. However, some of the chromosomes always appear imperfectly formed and attached to the remnant of the nucleolus. In several of these abnormal figures every chromosome, except those attached to the remnant of the nucleolus, is divided and well separated. It is probable that in some of these abnormal figures,

anaphase separation fails and a single nuclear membrane is formed about the entire diploid chromosome group.

Diploid cells in haploid callus tissues probably originate from binucleate cells (fig. 4, *B*). As previously noted, several binucleate cells were visible in haploid callus, but no evidence was obtained as to their method of formation. The nuclei of these cells always lay in contact with each other or in close proximity. In some cases, probably, the nuclear membrane broke along the lines of contact and the sister nuclei united.

MEIOSES IN POLLEN MOTHER CELLS AND EGG MOTHER CELLS OF HAPLONT *NICOTIANA GLUTINOSA*⁵

The prophase stages of the egg mother cells and pollen mother cells of haplont *Nicotiana glutinosa* are usually as regular as and very similar to those of diplont *N. glutinosa* or other species of *Nicotiana* (2).

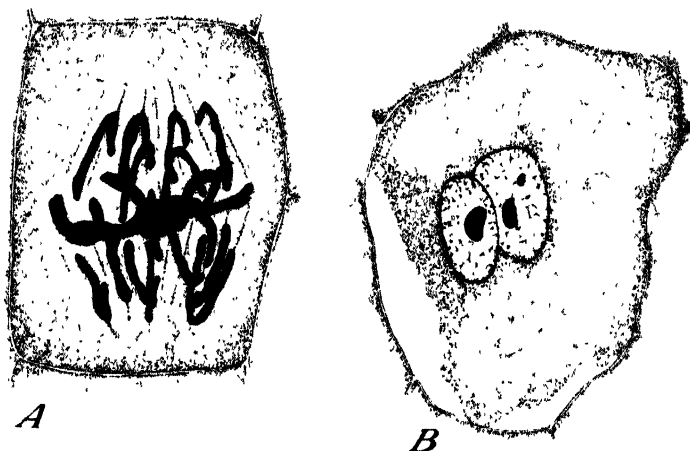


FIGURE 4.—Two cells indicating possible methods of chromosome doubling in *Nicotiana glutinosa* haplont 30.180-49: *A*, Root-tip cell in which a remnant of the nucleolus retards normal anaphase separation; *B*, binucleate callus cell, suggesting union of sister nuclei. $\times 4,750$.

The spireme of the haploid, however, is unpaired throughout the prophase (fig. 5, *A*). Hence the characteristic diploid ring and X-shaped diakinetic pairs are lacking in the haplont (fig. 6, *B*). During diakinesis in the haplont, 12 short, thick, rod-shaped univalents lie about the periphery of the nucleus.

Several twin ovules and binucleate egg mother cells and pollen mother cells are visible (pl. 2, *G*; fig. 5). The sister nuclei of these twin ovules and binucleate cells are always in the same stage of division and are haploid in nature. Binucleate cells usually have a single small chromatin granule in the cytoplasm. Although the origin of this granule is unknown, it is probably connected with that of the binucleate condition.

⁵ For convenience, the following symbols used in the University of California publications will be employed to designate the stages of meiosis: I and II, first and second meiotic divisions, respectively; I-M and II-M, first and second metaphase, respectively; I-A and II-A, first and second anaphase, respectively; and I-T and II-T, first and second telophase, respectively.

Since the binucleate cells occur in early meiotic prophase, they probably originate during an archesporial mitosis. Although direct evidence as to their mode of origin is lacking, several conditions indicate that they are not a product of extrusion of the nucleus from one germ cell to another (13, 23). These conditions are, briefly, as follows: (1) Binucleate germ cells are surrounded by an unbroken cell wall; (2) sister nuclei of binucleate pollen mother cells and egg mother cells lie far apart, thus apparently repelling each other; (3) it is extremely difficult, even in degenerate germ tissues, to separate the nucleus from the cytoplasm; and (4) egg mother cells are well separated by somatic tissue and air spaces, yet binucleate egg mother cells occur.

The remaining meiotic behavior is extremely irregular and variable. The 12 rod-shaped univalents contract until they are more or less spherical bodies. Immediately after the disappearance of the nuclear membrane a bipolar spindle is formed. The univalents are scattered over the spindle (pl. 3, A and B; fig. 6, D). They are approximately the same size as the II-M or univalent chromosomes of the diploid species.

One pollen mother cell is visible, with 2 achromatic figures and 12 univalents (fig. 5, B) scattered over each spindle. This pollen mother cell is probably an advanced stage of the binucleate prophase condition explained above.

Since the haplont does not form a I-M equatorial plate there is no foundation for orderly I-A distribution. The univalents are apparently distributed haphazard to the poles (pl. 3, C-E). All types of chance distribution from $0 < > 12$ to $6 < > 6$ occur. In many cases, however, univalents become laggards and go through an apparent division or fragmentation. This division usually fails and the univalent is finally distributed to a pole, or rarely left in the plasma. Now and then a laggard completes division, and the resulting parts

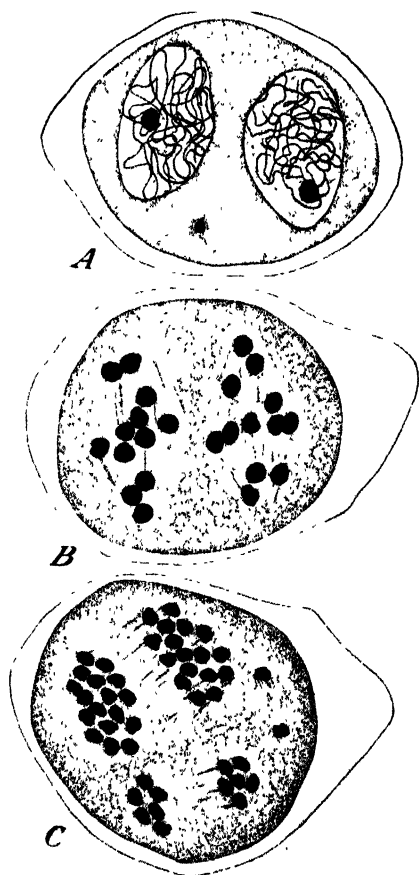


FIGURE 5.—Binucleate pollen mother cells in *Nicotiana glutinosa* haplont 30 180-49. A, Pachynema, showing spires of nuclei unpaired; B, I-M, in which each of the two achromatic figures contains 12 scattered univalents; C, II-A, in which the figure presumably

arose from a $17 < > 6$ I-A distribution following spindle fusion $\times 2,000$

are likewise included in the daughter nuclei or rarely left in the plasma. Since the products of such division are of various sizes, the division is undoubtedly a fragmentation.

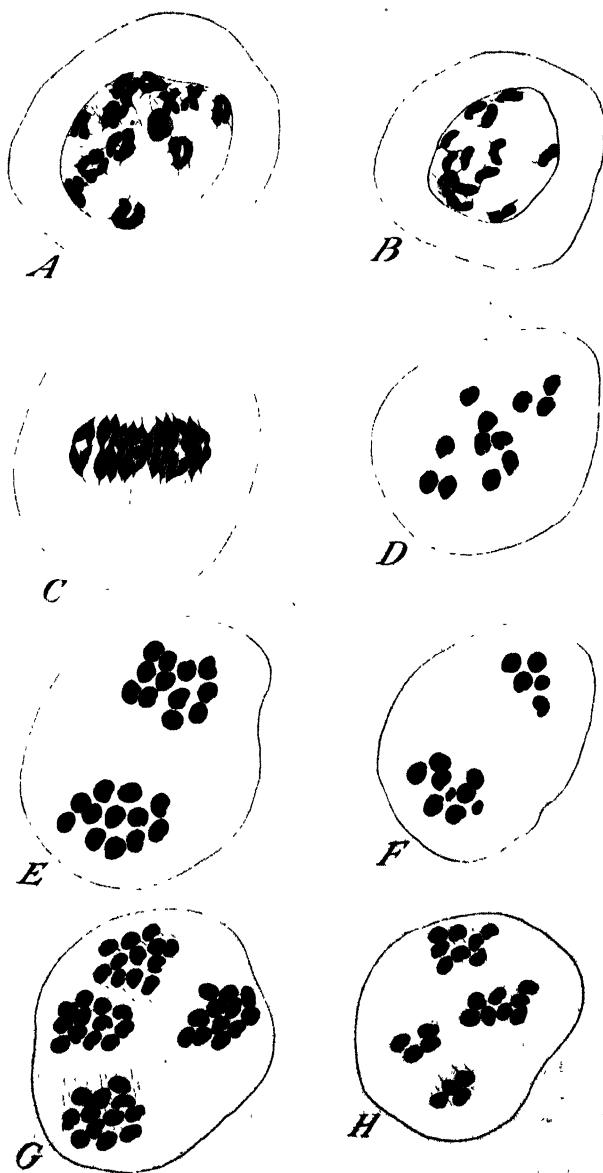
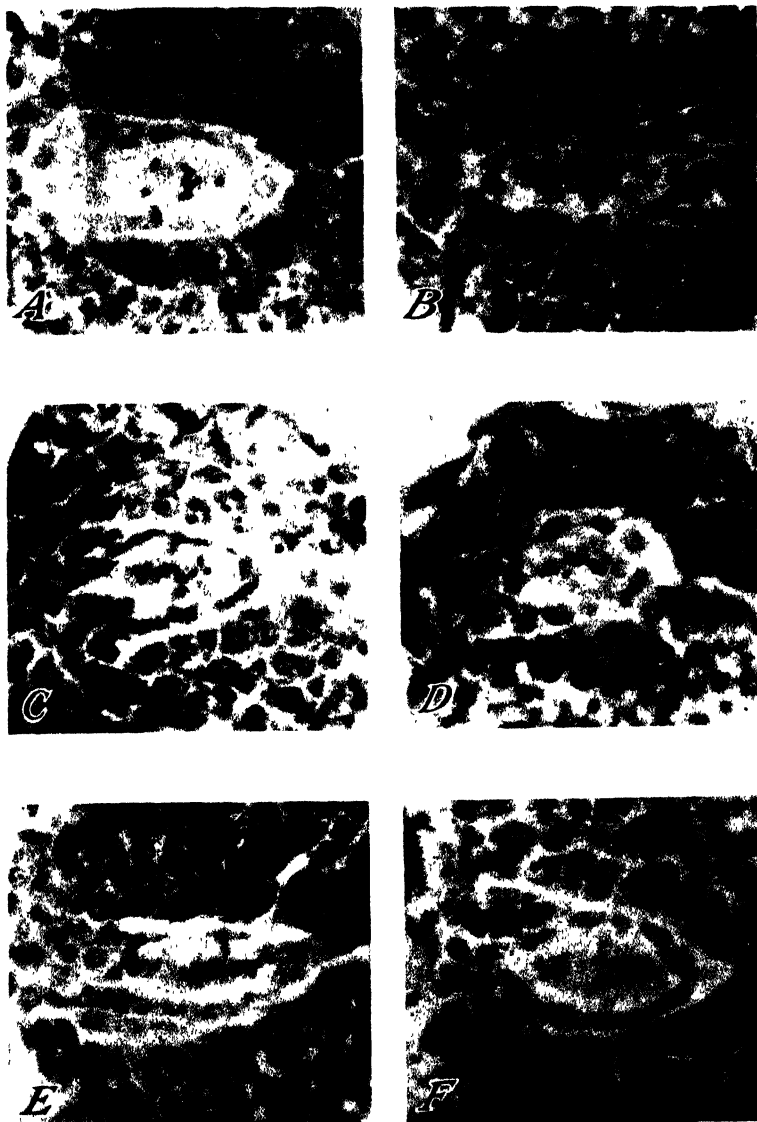
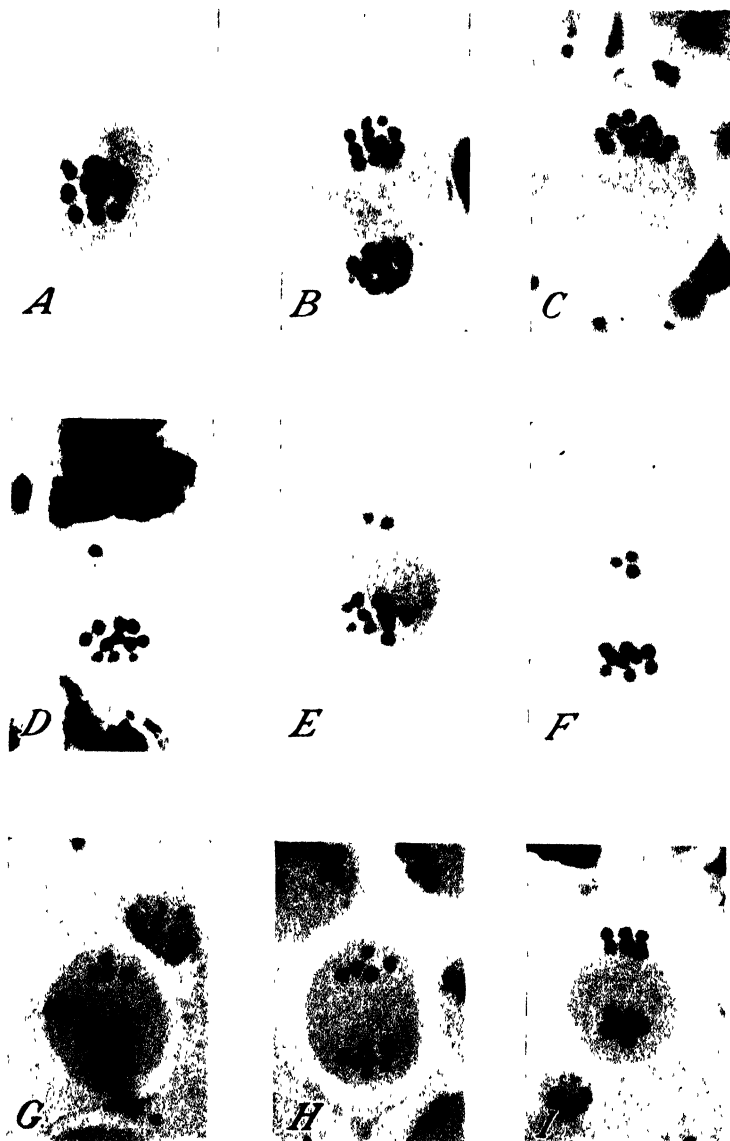


FIGURE 6.—Pollen mother cells of *Nicotiana glutinosa* diploint 30.005-1 (at left) and haploint 30.180-49 (at right): A and B, diakinesis; C and D, profile views of I-M; E and F, polar views of II-M, F showing 3 chromosome fragments; G and H, II-A. $\times 2,000$.

In general, interkinesis is very regular. The chromosomes at this stage are similar to those of the diploid state and exhibit the homo-



Egg mother cells of *Nicotiana glutinosa* haplont 30,180 49. A, Profile view of I-M equatorial plate, showing an extremely rare condition. B, Profile view of I-M showing a typical figure. C-E, Typical I-A figure. F, Typical II-M figure.



Nicotiana glutinosa. A and B, Diploid 30,005-1. A, Polar view I-M. B, Polar view II-M. C-I, Haplont 30,180-49; typical II-M figures, resulting from complete 0-12 to 6-6 I-A distribution; note relative size of pollen mother cells and chromosome of haplont and diploid.

typic split. The daughter nuclei of one pollen mother cell (pl. 2, *E*) are attached by a fine chromatin thread. This figure probably represents a suspended I-A or semiheterotypic division (21). As indicated in table 2, approximately 14.08 percent of the pollen mother cells show chromosomes or chromosome fragments in the plasma.

TABLE 2.—*Chromosomes or fragments in plasma of pollen mother cells at interkinesis*

Number of chromosomes or fragments	Pollen mother cells showing chromosomes or fragments in plasma	
	Number	Percent
0	177	85.92
1	19	9.22
2	6	2.91
3	3	1.46
4	1	.49
Total	206	100

As shown in table 3, the II-M plates exhibit various numbers of chromosomes. The table, expanded from 0 to 12, shows a mode of 6, as would be expected from I-A random distribution, and a mean value of 5.56. Typical II-M plates depicting complete random distribution are shown in plate 4, *C I*, and plate 3, *F*. Although the total number of chromatin units in the plates of each of these pollen mother cells is normally 12, it is occasionally less than this and rarely more. Out of 1,161 pollen mother cells (table 3) in which both plates were countable, 158, or 13.61 percent, showed chromosomes or chromosome fragments in the plasma. This percentage is in agreement with that of such chromatin units found in the plasma during interkinesis. The number of such elements in the plasma ranges from 1 to 4, as in the case of interkinesis. Fifteen, or 1.29 percent, of the 1,161 pollen mother cells (table 3) showed chromosome fragments within the II-M plates. Two of these pollen mother cells showed $3+1<>8+1$ fragments; 2 showed $4+1<>7+1$; 1 showed $4+1<>7+2$ (fig. 6, *F*); 9 showed $5+1<>6+1$; and 1 showed $5<1$ fragment $>6+1$.

It is to be noted (pl. 4, *C*) that II-M plates, which contain all 12 univalents, cannot be mistaken for I-M plates. Such II-M plates are always at the side of the cell or in the approximate position of one of the poles of the I-M spindle. Furthermore, such II-M pollen mother cells are always surrounded by other cells in the same stage of division.

The II-A chromosomes, which are slightly smaller than the II-M chromosomes, are about the same size as those of the diploid at this stage (fig. 6, *G* and *H*). In general behavior II-A is fairly normal. Occasionally, however, laggards occur. These laggards do not show the apparent division exhibited by the laggards in the case of I-A and apparently are ultimately included in one of the granddaughter nuclei. The majority of chromatin units left in the plasma during I are supplied with miniature spindles and divide at II-A. However, occasionally such a unit lacks a II spindle and fails to divide. Since the dividing units are considerably larger than the others, they probably are whole univalents. The chromatin units that fail to

divide and probably the laggards within the major II-A spindles are chromosome fragments.

TABLE 3.—Number of pollen mother cells showing chromosomes variously distributed on II-M plates

Type of distribution of chromosomes	Number of pollen mother cells showing chromosomes on—		Total number of pollen mother cells	Type of distribution of chromosomes	Number of pollen mother cells showing chromosomes on—		Total number of pollen mother cells
	Both plates ^a	Single plates ^b			Both plates ^a	Single plates ^b	
0	39	37	76	5	343	312	1,196
12	39	0	115	7	343	198	
1	48	58	156	6	237	348	822
11	48	2		6	237		
2	60	71	196				
10	60	5		Total	1,003	1,400	3,406
3	88	94	280		1,003		
9	88	10					
4	188	197	641				
8	188	68					

^a 158 pollen mother cells exhibiting chromosome fragments or chromosomes in the plasma are not included in the table

^b Plates exhibiting fragments are not included

The several counts of pollen mother cells at II-A were 5 of $\begin{smallmatrix} 7 \\ \diamond \\ 7 \end{smallmatrix}$ + $\begin{smallmatrix} 5 \\ \diamond \\ 5 \end{smallmatrix}$;

2 of $\begin{smallmatrix} 6 & 6 \\ \diamond & \diamond \\ 6 & 6 \end{smallmatrix}$; 1 each of $\begin{smallmatrix} 4 & 8 \\ \diamond & \diamond \\ 4 & 8 \end{smallmatrix}$ (fig. 6, II), $\begin{smallmatrix} 12 \\ \diamond \\ 12 \end{smallmatrix}$ + 0, $\begin{smallmatrix} 7 \\ \diamond \\ 7 \end{smallmatrix}$ + $\begin{smallmatrix} 1 \\ \diamond \\ 1 \end{smallmatrix}$ + $\begin{smallmatrix} 4 \\ \diamond \\ 4 \end{smallmatrix}$, and

$\begin{smallmatrix} 6 & 1 & 4 \\ \diamond & \diamond & \diamond \\ 6 & 1 & 4 \end{smallmatrix}$ + 1 + $\begin{smallmatrix} 1 \\ \diamond \\ 1 \end{smallmatrix}$.

The last of these pollen mother cells during I-A probably involved the following conditions: A random distribution of 4<>6; 1 univalent left in the plasma; and the fragmentation of 1 univalent, one part of which was included in the daughter nucleus with 4 univalents and the other part left in the plasma. Two exceptionally large pollen mother cells were visible. One of these (fig. 5, C) contained 2 groups of 17 chromosomes, 2 groups of 6 chromosomes, and 2 well-separated chromosomes in the plasma. This unusual pollen mother cell probably represents a more advanced stage of the binucleate condition, which, as previously explained, contains two I-M achromatic figures. The I-A distribution probably was 6<>6 and 11<>1. However, at one side of the pollen mother cell the adjacent spindle poles fused,

so that the distribution was $17 \begin{smallmatrix} > 6 \\ < \\ > 1 \end{smallmatrix}$. Normal homotypic division

followed, and the exceptional cell resulted.

As is indicated in table 4, the sporads⁶ were highly irregular. Since the spores were equivalent in size in only a few sporads, the segregation in the table is based entirely on the number of spores.

TABLE 4.—*Frequency of occurrence of microspores of various types*

Microspores	Number	Percent
Diads...	48	7.52
Triads...	18	2.82
Tetrads...	456	71.47
Above tetrads...	116	18.18
Total...	638	

The data in table 4 show that the majority of microsporads were tetrads. Although the spores of these tetrads were in the main unequal in size, a few were equal (fig. 7, *B*). The spores of the latter tetrads were considerably smaller than those of the diads (fig. 7, *D*) or of the tetrads of the diploid species (fig. 7, *A*). Obviously such spores contain less than the haploid chromosome complex and probably are the products of a uniform $6 < \geq 6$ or similar 1-A distribution.

Tetrads, as well as the other types of sporads, frequently contain micronuclei. Figure 7, *D* and *C*, show, respectively, a normal diad and a diad containing two small micronuclei. Approximately 30 to 40 percent of the diads contained such micronuclei. Possibly these diads resulted from a very irregular 1-A distribution, such as $1 < \geq 11$ or $2 < \geq 10$. If this was the case, then the adjacent products of daughter nuclei lay in such close proximity that they were included in one spore. A few of these micronuclei and the majority of micronuclei of other types of sporads undoubtedly resulted from chromosomes or fragments left in the plasma at I and II.

The spores of normal diads are approximately the same size as those of the tetrads of the diploid state. They probably arise from a $0 < \geq 12$ 1-A distribution and consequently contain a normal haploid chromosome complex.

Triads are possibly produced by a process similar to that by which diads containing micronuclei are produced. The sporads containing more than four spores undoubtedly result from highly irregular meiotic divisions or from binucleate pollen mother cells. Typical sporad types are shown in figure 7.

Up to II-T the behavior of the egg mother cells and pollen mother cells is practically identical. Possibly, however, the behavior of the egg mother cells is slightly more uniform and the mean 1-A distribution nearer the mode. However, following II-T, the majority of egg mother cells rapidly disintegrate to a deep-staining mass. As degeneration proceeds, the surrounding somatic cells elongate and gradually supplant the egg mother cells (pl. 2, *D*). After such replacement the somatic cells fail to develop further and finally the entire ovule disintegrates.

⁶ Although in the majority of plants the meiotic divisions produce a group of 4 spores, in numerous cases the divisions are highly irregular and from 1 to 12 or more spores are produced. Cases are known where 2 spores are characteristically formed. In literature dealing with these conditions such a statement as "the majority of tetrads contain only four spores" is rather common. It therefore appears that the term "tetrad" (*tetra* (Greek), four; and *ad* (Latin), toward) or "tetrad stage" is not expressive of the condition that obtains. The term "sporad" (*sporo* (Greek), seed, spore) is proposed for this stage or group of cells. Alternative terms, such as "sporocorymbus" (*corymbus* (Greek), cluster, group) are not in keeping with the terms "tetrad", "diad", etc., and are more cumbersome.

Although an occasional egg mother cell develops beyond II-T, such development is never normal and is rarely duplicated in another egg mother cell. The megasporads which develop always have at least two spores, the nuclei of which are approximately the same size as those of the microdiad. In addition to the large spores, there are occasionally one or two small ones. No definite evidence was obtained as to which spore develops into the megaspore and finally produces the female gametophyte. However, judging from the size of

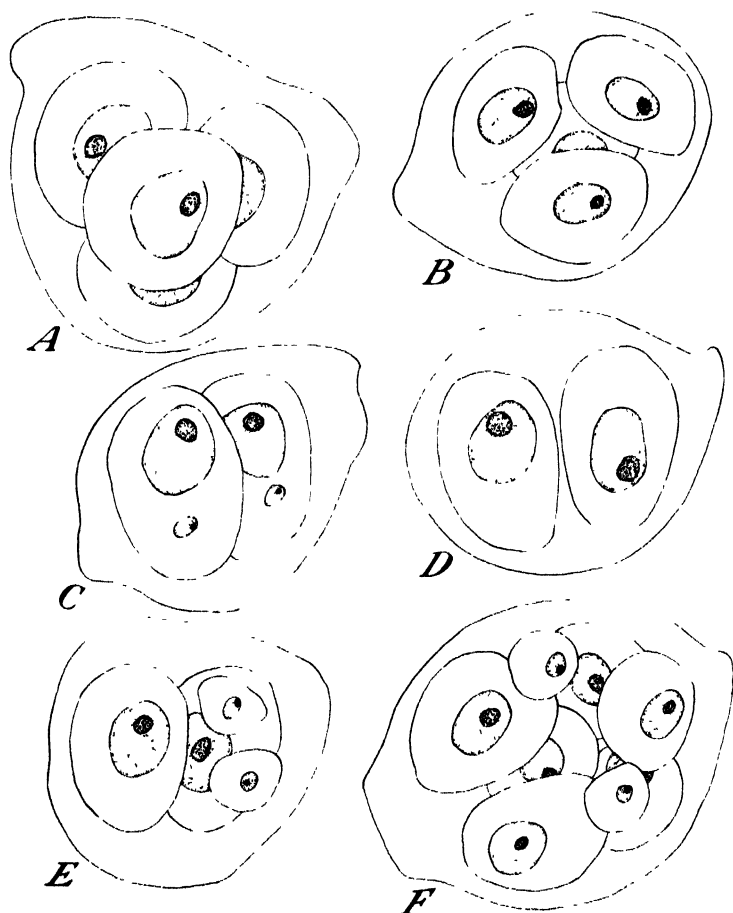


FIGURE 7 -Comparative microsporads of diploid (30,005-1) and haplont (30 180-49) *Nicotiana glutinosa*: A, Diploid tetrad, B-F, representative haplont sporads. $\times 2,000$.

gametophytic nuclei and from a few questionable chromosome counts of these nuclei, only spores containing approximately 12 chromosomes develop.

Further development is highly irregular and no definite stages occur. In the majority of cases, apparently, rapid division follows and from 4 to 7 nuclei are formed (pl. 2, D). Although in a few cases the nuclei are scattered throughout the gametophyte, no definite arrangement occurs. In the majority of cases the nuclei are in a single

compact group. Whether these nuclei remain in this condition until degeneration or whether they fuse is undetermined. However, later in development, the appearance of extremely large nuclei (pl. 2, *C*) indicates that the latter condition occurs. Such nuclei are visible in ovary material collected during anthesis. Although several of these large nuclei are in prophase, no gametophyte containing more than one such nucleus is visible. Counts of the latter nuclei indicate that they contain 35 or more chromosomes. Approximately at the time when fecundation should take place, degeneration starts, and all ovules, including those in which somatic cells have replaced the embryo sac, rapidly disintegrate.

As stated previously, no definite arrangement of stages occurs, hence no mature or normal embryo sac is formed. The most nearly normal condition is shown in figure 8, *A*. The three nuclei of this figure may represent the two synergids and the egg. In this case, the egg is extremely large in proportion to the synergids and may represent the union of several nuclei. Figure 8, *B* and *C*, shows two of the many irregularities.

Although no explanation is offered for these unusual figures, apparently in the one shown in figure 8, *B* and *A*, a polyploid somatic cell is replacing the embryo sac. Figure 8, *C*, represents a condition among well-advanced ovules and hence should be beyond the meiotic divisions. The nuclei at the bottom of the embryo sac likewise indicate an advanced stage. However, the 12 chromosomes

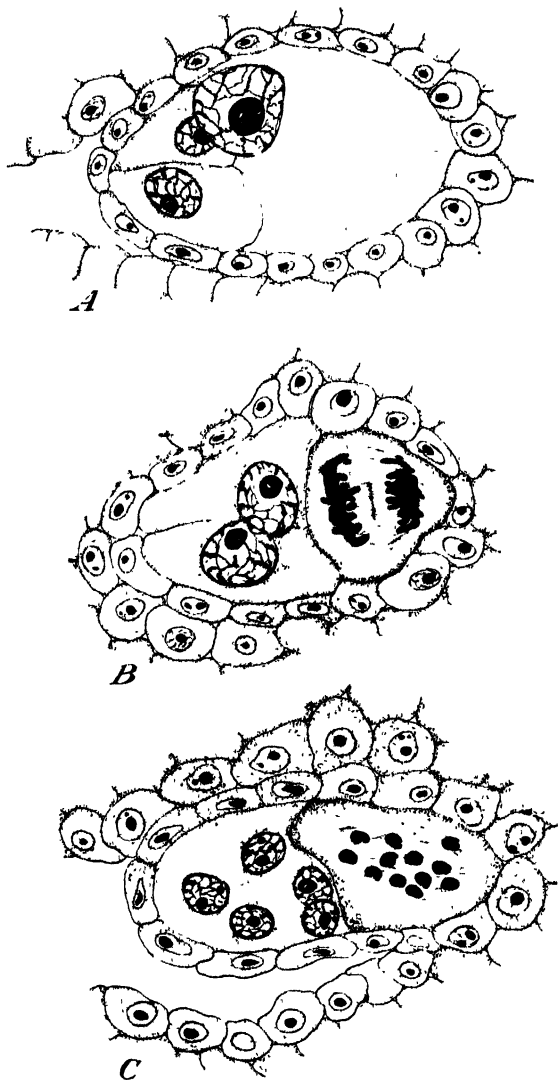


FIGURE 8.—A-C, representative female gametophytes of *Nicotiana glutinosa* haplont 30 180-49. $\times 950$.

in the upper part of the embryo sac are the size of univalents and have a decidedly meiotic form.

Since preparations of the male gametophyte are extremely difficult to obtain, no study of its development was made. However, the young pollen grains are extremely variable in size and shape, and only a few appear equivalent to those of the diploid species. During anthesis a few grains of the latter species were found in a whitish powder obtained from the anthers.

DISCUSSION

Inasmuch as the meiotic behavior of the *Nicotiana glutinosa* haplont is similar to that of other haplonts, especially in *Nicotiana*, this discussion will be confined mainly to somatic behavior and phenomena concerning such behavior. Moreover, since the literature dealing with haplonts has recently been reviewed by Gates and Goodwin (8) and by Kuhn (18), it will not be summarized here.

CHROMOSOME NUMBER AND MORPHOLOGY

That the normal somatic chromosome number is 24 (3) is confirmed by the present study of the chromosomes in diploid and haplont *Nicotiana glutinosa*. Since no pairing occurs during meiosis in haplont *N. glutinosa*, it is reasonably certain that 12 is the basic haploid number of chromosomes for the species.

Somatic chromosomes of *Nicotiana glutinosa* are similar in shape to those of *N. tabacum*, in which $n=24$ (23); to those of *N. alata*, in which $n=9$ (1, 22); and to those of *N. sylvestris*, in which $n=12$ (24). They are, however, considerably smaller in diameter than the chromosomes of these species and are not so variable in size. The chromosomes of *N. glutinosa* differ from those of *N. longiflora*, in which $n=10$ (14), in that the chromosomes of the latter species are uniformly rod-shaped. To some extent, therefore, specific chromosome distinctions separate the complex of *N. glutinosa* from the complexes of the other species mentioned.

According to the data and figures presented by the authors just cited, the chromosomes of *Nicotiana sylvestris* and *N. tabacum* are more nearly alike than those of any other of the species mentioned. In interspecific hybrids it has been shown that chromosome affinity is much greater in the *N. tabacum*-*N. sylvestris* combination than in any other combination of these five species⁷ (3; 4, p. 181; 7, p. 273-274; 9; 12).

COMPARISON OF DIPOIDY IN HAPLONT ROOTS AND TETRAPLOIDY IN DIPLOID ROOTS

As stated previously, the extent of diploidy is similar in root tips, marked roots, and root initials of haplont *Nicotiana glutinosa*. Only 2.21 percent of the haploid root tips gathered at random were partly diploid. This percentage indicates that the occurrence of diploidy in haplont roots is less frequent than is commonly believed (14, 17, 19, 23). In fact, diploidy in haplont roots does not appear to originate more frequently than does tetraploidy in diploid roots. Of the wholly diploid root tips of haplont *N. glutinosa*, 1.02 percent showed

⁷⁷ The degree of affinity between *Nicotiana alata* and *N. longiflora* seems to be questionable (7).

tetraploid chimeras. In a previous paper the writer (24) has shown that in diploid *N. sylvestris*, 2.50 percent of the root tips contain tetraploid chimeras, and he has reported a tetraploid chimera in *N. sanderae* Sander. Since 1930 the writer has observed such chimeras in *N. rusbyi* Britt. and *N. trigonophylla* Dun. Although in the last three cases no records were kept, it is certain that in each case tetraploidy originated as frequently as diploidy in haplont *N. glutinosa* root tips.

On the other hand, unless some phenomenon aside from frequent origin occurs, the high percentage (55.56) of wholly diploid root tips cannot be explained. The writer (24, p. 360) has stated:

The diploid cells of the haplonts are by far the most vigorous cells of the entire root tip and multiplication is more rapid in the diploid cells of such a root. The descendants of a diploid root initial in a haploid plant will in all probability persist in that meristem, and possibly in time dominate the tissues of the organ.

Such a change is precisely what happened in the *Nicotiana glutinosa* haplont 30.180-49. It has already been shown that the two large diploid chimeras in haploid root tips contain more $2n$ cells in the zone of cell division than in the zone of cell enlargement. It is also shown that the percentage of wholly diploid root tips increased from 0 to 55.56.

Such development and domination of diploidy in roots of haplont *Nicotiana glutinosa* is in direct contrast to that found in the case of tetraploidy in roots of diplont *N. sylvestris*. In the latter case the writer (24, p. 360) has stated:

That the larger sector * * * is gradually reaching its maximum size is indicated by the comparatively uniform decrease in the number of tetraploid cells from the region of elongation to the growing point. * * * If the root tip had been allowed to grow, this sector, and probably the majority of other sectors, would have become "islands" of tetraploid cells.

Hence, the size of $2n$ and $4n$ areas which develop from such initial cells in the roots of haplonts and diplonts is apparently determined by growth-rate relations of these tissues. Consequently such growth-rate distinctions must also determine the frequency of wholly $2n$ and $4n$ roots, since these roots must arise from such areas in the pericycle.

COMPARISON OF ROOT AND SHOOT IN RELATION TO POLYPOIDY

It is well known that diploidy in haplonts occurs more frequently in roots than in shoots (23, 15, 19). A similar condition exists in the case of tetraploidy in diplonts. Although the reason for the greater prevalence of these chimeral tissues in the root is apparently unknown, several clues are afforded by the nature of the root and shoot, especially by the relative amount of meristematic tissue in these organs and the relative degree of protection such tissues receive.

Undoubtedly the number of apical meristems in the root system is greater than in the shoot. Meristematic tissue in the shoot is covered with a more effective protective tissue than is that in the root. Moreover, the apical meristem of the shoot receives additional protection from young leaves and is therefore better shielded from environmental changes that may initiate polyploidy. Hence, it seems probable that polyploidy originates more frequently in the root than in the shoot.

Although the environmental variations to which the shoot is subjected are ordinarily greater than those that affect the root, as great

or greater variations are usually encountered by roots grown for cytological investigations. Such roots, generally from potted plants that soon become more or less pot-bound, are subjected to various and sudden changes in temperature and moisture. Since the root is not so effectively constructed as the shoot to endure such variations, the greater frequency of polyploidy in roots grown under such conditions is not surprising.

The behavior of haplont *Nicotiana glutinosa* is in harmony with the foregoing facts. It was shown in a preceding section that all roots gathered from field plants or from plants (cuttings) grown in an ample volume of soil were wholly haploid. On the other hand, as soon as the plants become more or less pot-bound, diploidy suddenly occurred.

It is probable, however, that tetraploid and also diploid chimeras are of more frequent occurrence in shoots than has been observed, since apical meristems of shoots are very seldom examined.

HAPLONT CALLUS TISSUE IN RELATION TO DIPLDIDY

As previously shown, the callus at the proximal ends of shoot cuttings of haplont *Nicotiana glutinosa* originates from the vascular cambium and is composed chiefly of haploid cells, although a few diploid and binucleate cells occur. The mode of origin is in agreement with that reported by Priestley and Swingle (20). The occurrence of diploid and binucleate cells in the proximal callus is probably due to the effect of environmental changes on the unprotected dividing cells. Such diploid cells have no effect on the composition of roots produced by cuttings, since new roots originate from the vascular cambium several sections above the callus.

No distal callus is formed in haplont cuttings of *Nicotiana glutinosa*. However, distal callus similar in composition to the proximal callus of haplont cuttings of *N. glutinosa* has been reported by Jørgensen (16) and by Lindstrom and Koos (19). The occurrence of diploid cells in the callus of decapitated haplonts may be attributed to causes similar to those resulting in diploid cells in proximal callus. However, unlike the diploid cells of the proximal callus, those of the distal callus may have a profound effect on the composition of new tissues. Since Priestley and Swingle (20) found that buds are exogenous in origin, whereas roots are endogenous, the occasional occurrence of diploid and tetraploid shoots from decapitated haplonts may be accounted for by the chimeral composition of the distal callus.

MEIOTIC FEATURES OF HAPLONT NICOTIANA GLUTINOSA

As previously stated, the meiotic behavior of haplont *Nicotiana glutinosa* is very similar to that of other *Nicotiana* haplonts (2, 5, 6, 11, 17, 23). Because of this similarity and since Hollingshead (14) has recently discussed the meiotic resemblances and differences of haplonts, it appears unnecessary to present any further compilations here. Hence, the new, unusual, and important meiotic and gametophytic features of the *N. glutinosa* haplont are merely enumerated. These are as follows:

* The occurrence of binucleate egg mother cells and pollen mother cells and of twin ovules during the meiotic prophase.

The complete lack of any observable conjugation during meiosis.

The lack of the homotypic split or apparent equational division of univalents during I.

The apparent fragmentation of univalents during I-A.

The formation of two I-M achromatic figures in binucleate germ cells.

The success of I-A random distribution, the 12 univalents being distributed, in nearly all cases, in all types of distribution from $0 < > 12$ to $6 < > 6$.

The very rare occurrence of semiheterotypic divisions.

The II-M mode of 6 and mean value of 5.56.

The occurrence of chromosome fragments at II-M.

The fairly normal II-A distribution.

The occurrence of II-A pollen mother cells containing 48 chromosomes, which probably represent advanced stages of the binucleate condition. The possible fusion of the I-M achromatic figures in binucleate cells, giving rise to II-M and II-A plates containing more than 12 chromosomes.

The occurrence of highly abnormal sporads and occasional diads, the spores of which contain the complete haploid chromosome complex.

The disintegration of the majority of egg mother cells following II-T, and their gradual replacement by somatic cells.

The formation of an occasional female gametophyte.

The great irregularity and lack of definite stages in the female gametophyte.

The apparent fusion of nuclei in the female gametophyte and the occurrence of polyploid nuclei.

The degeneration of all ovules at the approximate time when fecundation should occur.

The occurrence of a few apparently normal pollen grains.

SUMMARY

Two additional haplonts of *Nicotiana glutinosa* were found during the spring of 1930 in the botanical gardens of the University of California. One of these plants is described and figured.

The somatic chromosome complex of *N. glutinosa* consists of 24 chromosomes. The basic haploid number is apparently 12.

The chromosomes of the somatic garniture exhibit morphological differences permitting the recognition of nine distinct types.

Diploidy frequently occurs in the root system of haplont *N. glutinosa*. The percentage of wholly diploid root tips observed increased from 0 at 22 weeks to 55.56 at 37 weeks. Diploid areas were present in 2.21 percent of the haploid root tips, and 1.02 percent of the diploid root tips contained tetraploid areas.

Diploid areas extending from the zone of cell division into the zone of cell enlargement contain more cells in the former zone and hence are larger than in the latter zone.

Marked diploid and haploid roots produced wholly diploid and wholly haploid tips, respectively.

Of 19 pieces of rootlets, 13 had wholly diploid root initials; 5 had wholly haploid initials; and 1 had 1 haploid and 2 diploid initials.

The basal callus tissue of cuttings is composed mainly of haploid cells, although a few diploid and binucleate cells occur.

Root initials in the callus originate several sections above the callus. All initials are wholly haploid.

Out of 6 cuttings, only 1 had diploid root tips in its early history.

Chromosome doubling in root tips is possibly due to the failure of anaphase separation, following the retention of the nucleolus to this stage. Chromosome doubling in callus tissue is possibly due to the fusion of the nuclei of binucleate cells.

Meiotic and gametophytic behavior is exceptionally irregular, and several new and unusual features occur. The term "sporad" is suggested for the spore or group of spores (tetrads, triads, microcytes,

etc.) resulting from meiotic divisions. A summary of meiosis is given in the final section of the discussion.

Evidence is presented that the high percentage of diploidy in haplont roots of *N. glutinosa* is due to the relative growth relations of $2n$ and n tissues rather than to the frequent origin of $2n$ initials.

Growth rates of $4n$ and $2n$ tissues in relation to the occurrence of tetraploid chimeras in diplonts are discussed.

Evidence is presented that diploidy in haplont roots is probably due to the effect of frequent and sudden environmental changes on poorly protected meristematic tissue.

Evidence is presented that the basal-callus tissue of cuttings forms a protective coat for adventitious root initials. Comparisons are drawn between the basal callus and root initials, and the conditions presumably existing in the apical callus and bud initials.

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LIVER-SPOT DISEASE OF PECAN FOLIAGE CAUSED BY GNOMONIA CARYAE PECANAE, NOV. VAR.¹

By J. R. COLE²

Associate pathologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In the vicinity of Shreveport, La., during the early summer, a leaf spot may be found on the foliage of the pecan (*Illicia pecan* Brit.). This type of leaf spot was first reported on the pecan by Demaree,³ who found it in Louisiana and Texas and called it "western sooty spot." The color of the spot is first dresden⁴ and later cinnamon brown, but never black or sooty. It is therefore suggested that the name be changed to "liver spot" as being more descriptive of the disease.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The liver-spot disease has been under observation by the writer for several years, and during the season of 1931 an extensive survey was made to determine its distribution and its economic importance to the pecan industry. At that time the disease was prevalent in central Mississippi, Arkansas, northern Louisiana, and Texas. Unlike most other parasitic diseases of the pecan, it seems not to thrive in regions of high humidity, especially near the Gulf coast of Louisiana and Mississippi, and it was not found in southern Alabama, southern Georgia, or northern Florida during July 1931 when some orchards of these States were visited by the writer.

Liver spot is not considered to be as serious as some other parasitic diseases of pecans, especially scab caused by *Cladosporium effusum* (Wint.) Demaree, but it is severely damaging to pecan foliage in many localities in the southwestern part of the United States. Since it is sometimes accompanied by other leaf spots, especially downy spot caused by *Mycosphaerella caryigena* Demaree and Cole, and vein spot caused by *Leptothyrium nervisedum* Cole, it is difficult to estimate accurately the damage due to this disease.

The fungus attacks trees of low vitality, particularly those thickly crowded or growing in soil low in humus; it also attacks vigorous trees growing in the most fertile soil of the Red River Valley. During the early fall of 1930 and 1931 some of the leading varieties of pecan in western Louisiana and eastern Texas were prematurely defoliated by the liver-spot pathogene.

DESCRIPTION OF THE DISEASE

The disease first appears on the lower surface of the leaflets, usually along the midrib (fig. 1), as umber or dresden-brown circular dots,

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² The writer acknowledges his indebtedness to C. L. Shear, Division of Mycology and Disease Survey, for assistance in the classification of the causal organism; to John W. Roberts and J. B. Demaree, Division of Fruit and Vegetable Crops and Diseases, for criticism of the manuscript; and to Edith K. Cash, Division of Mycology and Disease Survey, for assistance in translating the technical description into Latin.

³ DEMAREE, J. B. DISEASES OF PECANS IN THE SOUTHERN STATES. U.S. Dept. Agr. Farmers' Bul. 1672, 28 pp., illus. 1931.

⁴ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D.C. 1912.

resembling the color of liver. These dots enlarge and may reach a diameter of 1 cm, rarely more, except where they coalesce. The color

remains the same until late summer or early fall, when it changes gradually from Dresden to cinnamon brown and pycnidia appear in the central parts of the diseased areas (fig. 2). At this time the diseased areas become visible on the upper surface of the leaflets. Figure 3 shows a leaflet with about two thirds of its surface discolored by the fungus and the lower edge darkened by the masses or groups of pycnidia. At this stage of the disease the leaflets usually are shed.

THE CAUSAL FUNGUS

CONIDIAL STAGE

During late summer, when the diseased areas change from Dresden to cinnamon brown, groups of reddish-brown pimplelike pycnidia form in the center of the spots. The production of pycnidia may continue until the entire spot is covered. They are at first subcuticular, later superficial. The pycnidia are not firmly embedded in the host tissue, as the outer covering begins at the top of the epidermal cells. The pycnidial covering consists of very small, irregularly shaped cells, which have thin walls that are difficult to distinguish clearly without the aid of high magnification. This covering varies in thickness from 2 to 3 cells at the base to 4 to 6 cells at the top. There is no

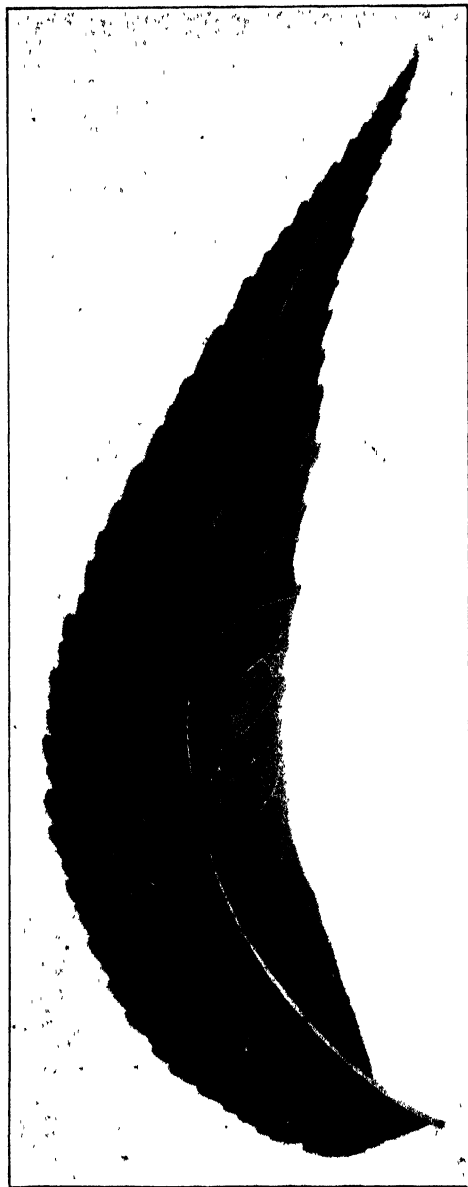


FIGURE 1.—Peran leaf showing early stages of liver spot.

ostiole. The base of the pycnidium is made up of stromatic layers, 2 to 4 cells thick, which apparently dissolve the epidermis. From these stromatic cells hyphae extend into the spongy parenchyma of the host (fig. 4, B). The conidia are borne at the apical end of short conidi-

ophores, which are slightly longer than the conidia. The conidiophores are simple, hyaline, and 1 to 2 septate. The conidia are continuous, hyaline, curved, fusoid, large at the basal end, and attenuated toward the apex.

PERITHECIAL STAGE

In the vicinity of Shreveport, La., perithecia begin to develop in late winter and reach maturity in the early spring. They develop mostly on the lower surface of the leaves, among the old pycnidia, most often singly, but sometimes in groups of twos or threes. The perithecia, which are embedded deep in the host tissues, extend through the spongy parenchyma and penetrate the palisade cells (fig. 4, *E*). The long beaks of the perithecia, which have well-developed ostioles, extend above the leaf and may be seen with the unaided eye. The perithecia are somewhat flask-shaped and are composed of thick-walled cells. The perithecial walls are dark brown, 2 cells thick at the bottom and top, and 3 to 4 cells thick on the sides.

The asci are hyaline, eight-spored, and cylindrical; they are not accompanied by paraphyses. At the apical end they have a pore, which is characteristic of the genus *Gnomonia*. The ascospores are straight or curved, hyaline, guttulate, and one-septate, both cells being about equal in size, with a slight constriction at the septum. The spores are enveloped in a gelatinous substance without defined cell walls and with pointed hyaline appendages at each end, the combined length of two appendages being about equal to the length of the spore. Before the spores are discharged from the ascus, the appendages lie close alongside the spores (fig. 4, *D*), extending after the discharge of the spores.

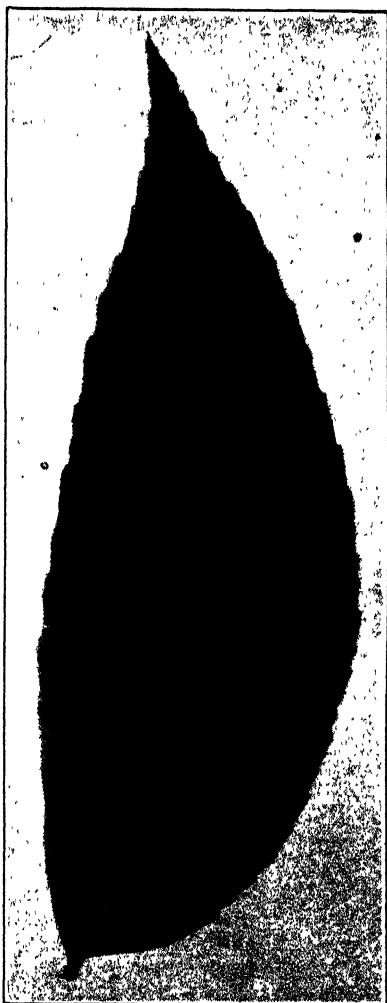


FIGURE 2.—Pecan leaf showing both early and late stages of liver spot. Fruiting bodies are appearing in the lighter areas.

IDENTITY OF THE FUNGUS

Ellis and Everhart⁵ described the imperfect stage of a fungus on hickory leaves in 1891 as *Gloeosporium caryae*. Ellis and Everhart⁶ re-

⁵ ELLIS, J. B., and EVERHART, B. M. NEW SPECIES OF FUNGI FROM VARIOUS LOCALITIES. *GLOEOSPORIUM CARYAE* ELL. AND DEARNES. Acad Nat. Sci., Phila. Proc. 1891: 82. 1892.

⁶ ELLIS, J. B., and EVERHART, B. M. THE NORTH AMERICAN PYRENOMYCETES, A CONTRIBUTION TO MYCOLOGIC BOTANY, p. 326, Newfield, N.J. 1892.

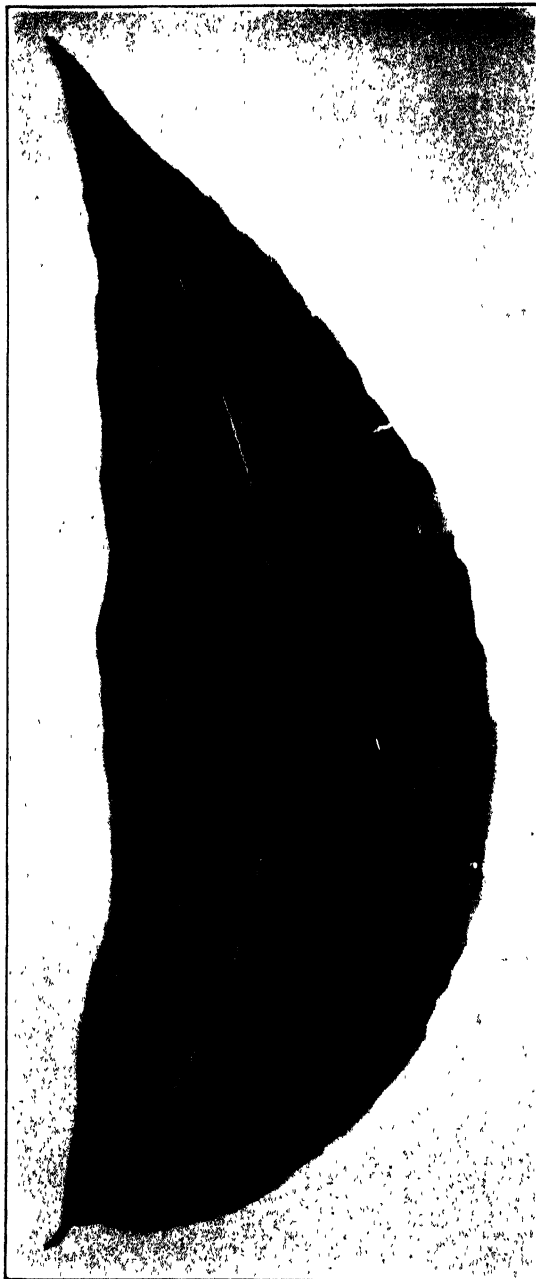


FIGURE 3.—Pecan leaf showing advanced stage of liver spot. Midrib and veinlets are dying; fruiting bodies are abundant. Leaves are usually shed soon after reaching this stage.

ported *Gnomonia setacea* (Pers.) Ces. and De Not., var. *macrospora* Ell. and Ev., as occurring on fallen leaves of *Quercus*, *Castanea*, and *Carya* (*Hicoria*) near Newfield, N.J. Matz⁷ reported a two-spored *Gnomonia* on living pecan leaves in Florida in 1917. Wolf⁸ collected, in the vicinity of Auburn, Ala., an imperfect fungus on hickory leaves, which he called *Gloeosporium caryae* Ell. and Dearn., and by placing the leaves outdoors over winter, he found the perfect stage the following spring. This perfect stage he described as *Gnomonia caryae*. So far as the writer has been able to determine, Demaree⁹ was the first to collect the imperfect stage of the liver-spot fungus on pecan leaves. He considered the causal organism to be *Gloeosporium caryae* var. *curvisporum* Dearn.

In the early studies of the liver-spot pathogene, the general morphological characteristics of the fungus indicated that it should be classified as belonging to the genus *Gloeosporium*. Since *Gloeosporium caryae* is common on hickory leaves in Louisiana and Mississippi, collections of hickory leaves bearing the fun-

⁷ MATZ, J. AN UNDESCRIBED GNOMONIA ON PECAN LEAVES. Fla. Agr. Expt. Sta. Ann. Rpt. 1917: 89R-94, illus. 1918.

⁸ WOLF, F. A. A NEW GNOMONIA ON HICKORY LEAVES. Ann. Mycol. 10: 488-491, illus. 1912.

⁹ DEMAREE, J. B. See footnote 3.

gus were made in different parts of these States, and type specimens of *G. caryae* were secured from the herbarium in Washington, D.C. Microtome sections of the hickory and the pecan leaves showed that the fruiting bodies of what has been called *G. caryae* on hickory leaves and the imperfect stage of the liver-spot fungus on pecan are not acervuli but are pycnidia (figs. 4-7). The cell structures in the covering of the pycnidia are quite distinct under high magnification. C. L. Shear, of the

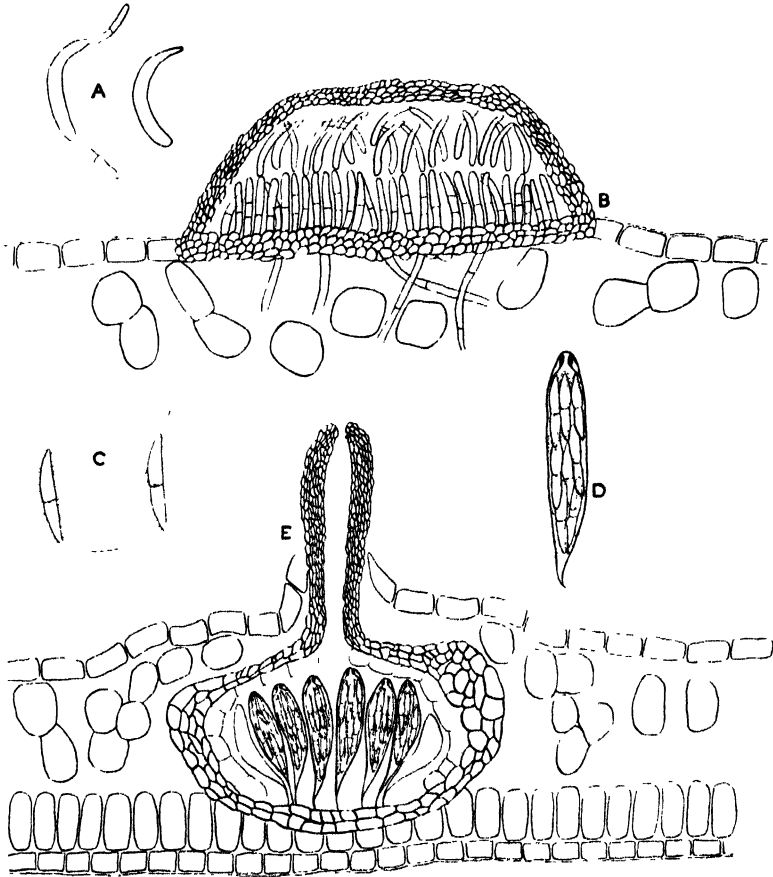


FIGURE 4.—The liver-spot fungus on pecan: A, Types of conidia and germination ($\times 1,800$); B, pycnidium ($\times 900$); C, types of ascospores and germination ($\times 1,200$); D, ascus with eight ascospores ($\times 900$), E, perithecium embedded in leaf ($\times 600$).

Bureau of Plant Industry, United States Department of Agriculture, examined the microtome sections and suggested that the imperfect stages belong in the genus *Leptothyrium* and not *Gloeosporium*. A comparison of the imperfect fungus on pecan and that on hickory leaves showed that the pycnidia of the liver-spot fungus on pecan commonly appear in groups, whereas on the hickory they are usually scattered over the entire leaf. The pycnidial covering of the liver-spot fungus is 2 to 6 cells thick, whereas the covering on the hickory fungus is only 1 to 2 cells thick. The conidia of the liver-spot fungus are long and slender (15μ to 20μ long and 2μ to 3.5μ wide); those of the hick-

ory fungus are shorter (7μ to 10μ long and 2μ to 3.5μ wide). Wolf reported the latter conidia to be 7μ to 10μ long and 1.5μ to 2μ wide.

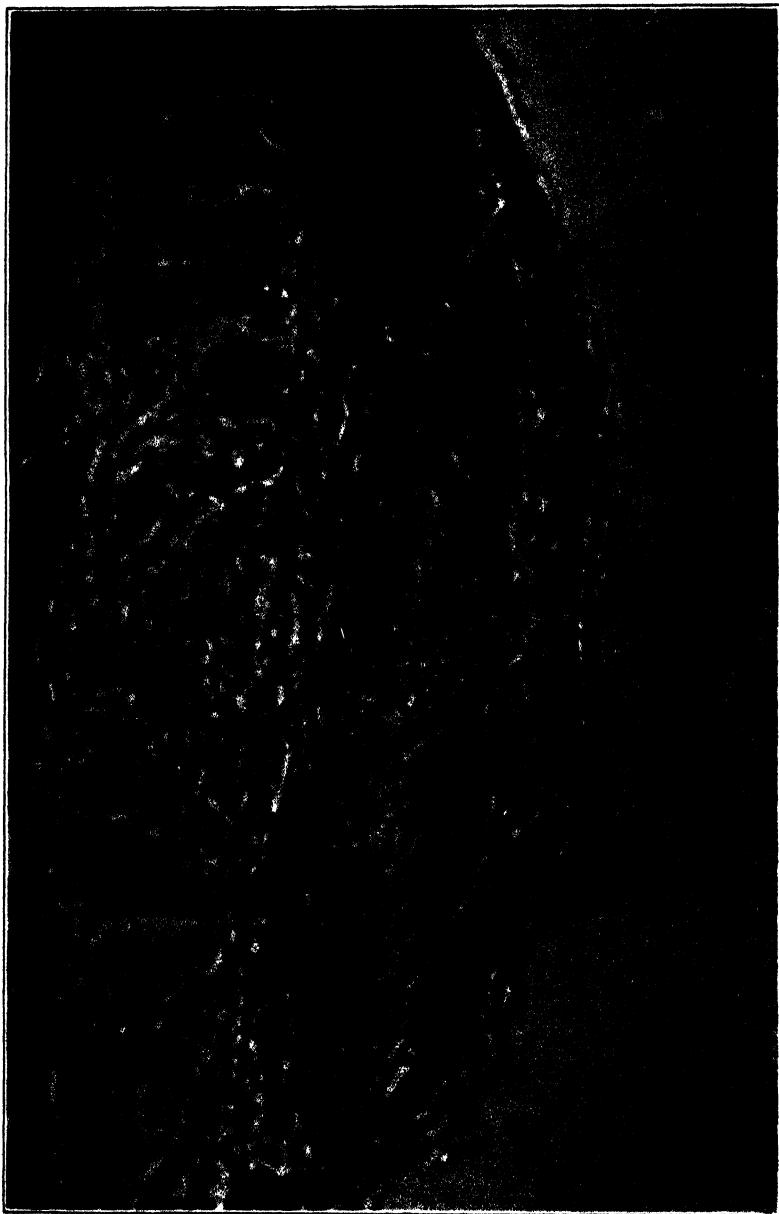


FIGURE 5.--Photomicrograph showing conidial stage of the liver-spot fungus on pecan. The pycnidial covering is composed of several layers of cells ($\times 1,400$).

In making cross-inoculations with the conidia from pecan or from hickory, the writer was never able to get as high a percentage of

infection by inoculating hickory with the conidia from pecan, or vice versa, as by inoculating either species with conidia from the corresponding host.

Type specimens of *Gloeosporium caryae* var. *curvisporum* when examined appeared distinctly different, both macroscopically and microscopically, from the imperfect forms of liver spot and the fungus

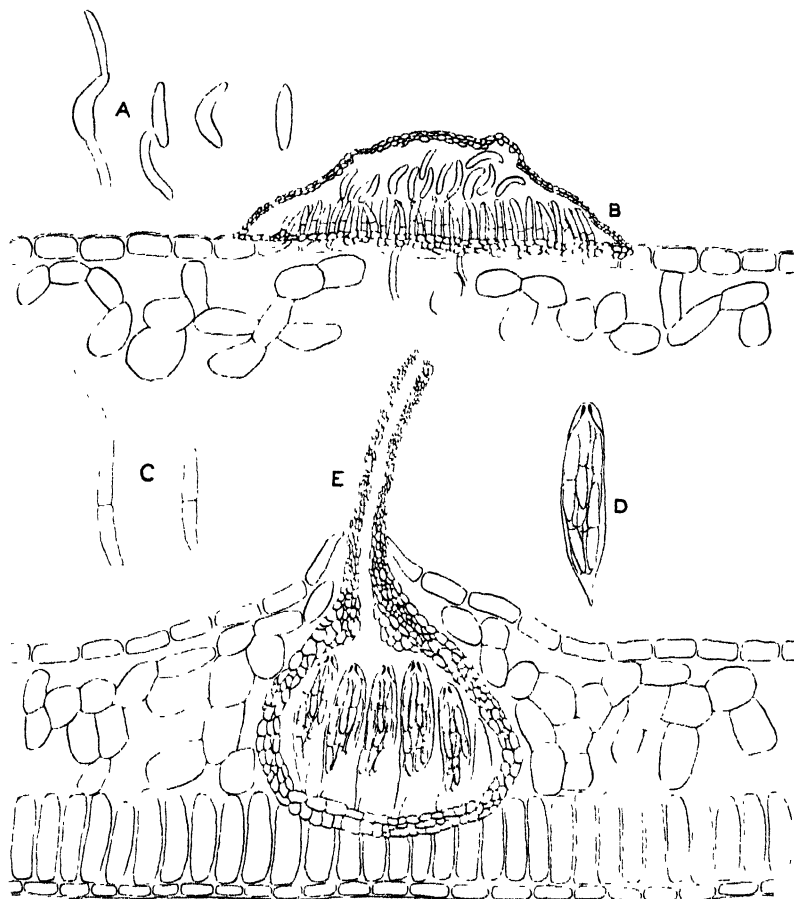


FIGURE 6.—*Gnomonia caryae* Wolf on hickory: A, Types of conidia and germination ($\times 1,800$); B, pyrenidium ($\times 900$); C, types of ascospores and germination ($\times 1,200$); D, ascus with eight ascospores ($\times 900$); E, perithecium embedded in leaf ($\times 600$).

on hickory. They conform more to the *Gloeosporium* than to the *Leptothyrium* type.

Because of the fact that there is a morphological similarity between the imperfect stages of the liver-spot fungus and the hickory fungus, and because the inability to get a high percentage of infection by cross-inoculating the fungi may have been due to physiologic strains rather than separate species, the writer suggests that the imperfect fungus on hickory be called *Leptothyrium caryae* and that on pecan *L. caryae* var. *pecanae*. Both *L. caryae* and *L. caryae* var. *pecanae* are different from *L. nervisedum* which occurs on the foliage of pecan.

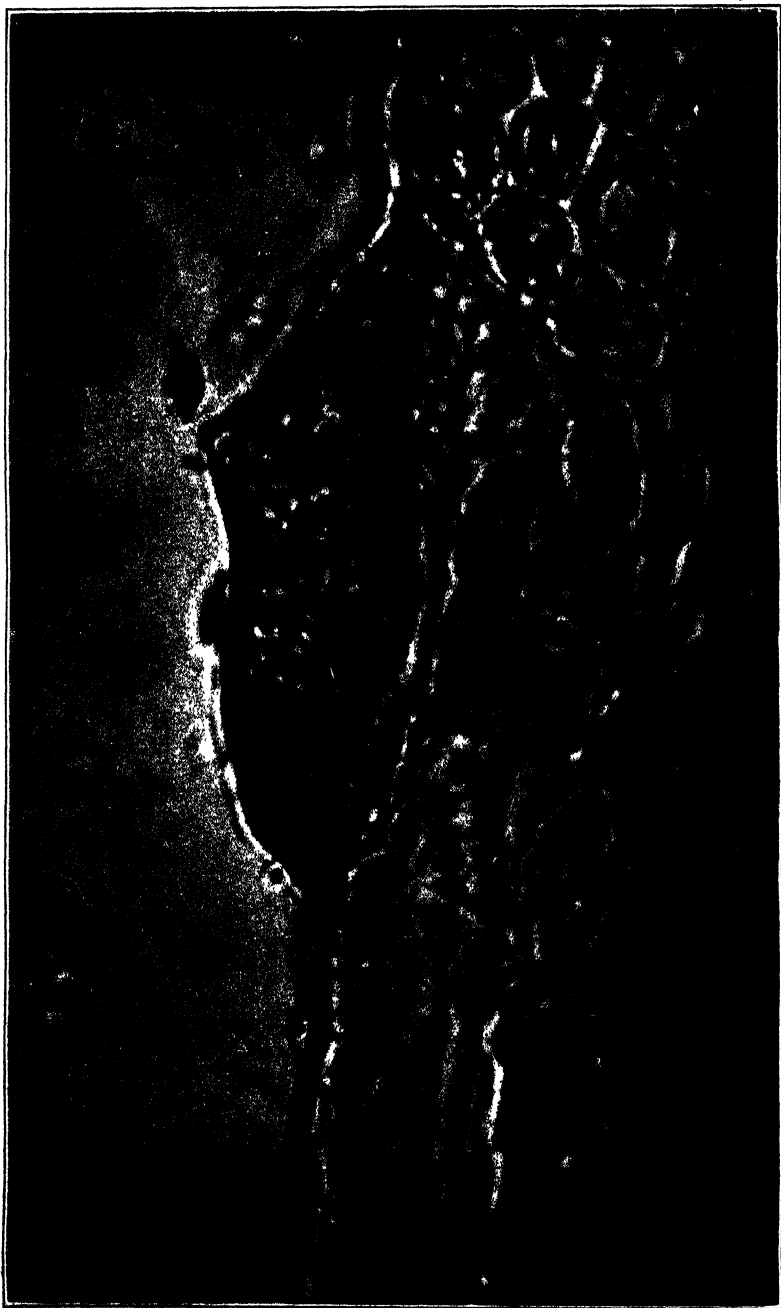


*FIGURE 7.—Photomicrograph showing conidial stage of *Gnomonia caryae* Wolf on hickory. The pycnidial covering is composed of one or two layers of cells ($\times 1,400$).

The last-named fungus confines its attacks to the vascular system of the leaves, and it differs in morphological characters from the other two fungi. Its pycnidial covering is dark brown to black, its cells are indefinite, and its conidia are oblong to ovate. In cultural characters also it differs from the other pathogenes.

Pecan leaves bearing pycnidia of the liver-spot fungus were placed in cages outdoors to overwinter in the vicinity of Shreveport, La. Examinations in the early spring disclosed mature perithecia among the old pycnidia. Because of the morphological characteristics of the perithecia, the fungus was placed in the genus *Gnomonia*. The spores of this fungus have appendages and, since Ellis and Everhart¹⁰ reported a *Gnomonia* on *Carya* (*Hicoria*) having spores with appendages, a comparison of the two fungi was made.

An examination of type specimens of *Gnomonia setacea* (Pers.) Ces. and DeNot. and *G. setacea* var. *macrospora* disclosed the fact that the ascospores either were immature or had deteriorated so much that a fair comparison could not be made. The former is found on pecans and oaks in the South and the latter on hickory in the East, especially in New Jersey. The perithecia of these organisms are not confined to any part of the leaflets, but are scattered over the entire leaf surfaces. There is no evidence of old pycnidia among the perithecia, nor has there been a conidial stage connected with either organism, so far as the writer has been able to ascertain. The descriptions of *G. setacea* and *G. setacea* var. *macrospora*, according to Ellis and Everhart,¹⁰ indicate that these forms are distinct from *G. caryae* Wolf on hickory, being 4- to 8-spored and septate in the middle without constriction. During the studies of *G. caryae* and the liver-spot fungus, the writer has never observed an ascus with less than eight spores, and the spores also show a slight constriction at the septum (figs. 4 and 6). Furthermore, the spores of both of these forms are larger than those of either *G. setacea* or *G. setacea* var. *macrospora*. Matz¹¹ also described a *Gnomonia* on pecan, but since that fungus produces its fruiting bodies on living leaves and since two spores, rarely three or more, are produced in an ascus, it is considered a species distinct from both *G. caryae* and the liver-spot fungus.

The morphological characteristics of Wolf's *Gnomonia* on hickory and those of the fungus on pecan, herein described, are similar, except that the spores of the latter are usually accompanied by appendages composed, perhaps, of a gelatinous substance without a defined cell wall. Appendages were not reported on the spores of *G. caryae* by Wolf, nor were they found by the writer (fig. 6, C). The spores of *G. caryae* are longer than those of the liver-spot fungus; the former are 25μ to 33μ long and 5μ wide, whereas the latter are 22μ to 28μ long by 3μ to 4μ wide. The measurements of the pecan spores do not include the appendages.

After a number of unsuccessful attempts to get infection with ascospores from *Gnomonia caryae* and the liver-spot fungus, inoculations were made with conidia from the ascospore cultures. As previously stated, a higher percentage of infection was obtained when the inoculum was placed on the host species (hickory and pecan, respectively) than when cross-inoculations were made.

¹⁰ ELLIS, J. B., and EVERHART, B. M. (See footnote 5.)

¹¹ MATZ, J. (See footnote 7.)

The growth characters of the two fungi on artificial media were very much alike. However, conidia were produced in pycnidia when cultured from either the imperfect or the perfect stage. Wolf¹² found that when cultures were made from the conidial stages of *Gnomonia caryae* the conidia were not borne in acervuli but on hyphae or short conidiophores. The writer also found this to be true.

Because of the similarity between *Gnomonia caryae* and the liver-spot fungus, the writer suggests that the latter be designated *Gnomonia caryae* var. *pecanae*.

TECHNICAL DESCRIPTION

Gnomonia caryae var. *pecanae*, nov. var.

Peritheciis hypophyllis, pezizoideis, singulis vel 2-3 gregariis, plerumque inter pycnidia sparsis, sine rostris 300 μ -350 μ latis, 150 μ -250 μ altis; rostris 200 μ -250 μ longis, 50 μ -60 μ latis; pariebus perithecialibus apice et infra 2, lateribus 3-4 stratis cellularum compositis, atrobrunneis; ascis hyalinis, octosporis, poro apicali et pariebus tenuibus praeditis, cylindraceis, 45 μ -50 μ longis, 6 μ -8 μ latis; ascosporis rectis vel curvatis, hyalinis, guttulis, uniseptatis, utrinque setosis, 22 μ -28 μ longis, 3 μ -4 μ latis.

Pycnidiiis superficialibus, subgloboseis, rufo-brunneis, 75 μ 300 μ latis; conidiis continuis, hyalinis, curvatis, fusiformibus, versus apicem attenuatis, 15 μ -20 μ longis, 2 μ -3.5 μ latis, conidiophoribus simplicibus, hyalinis, 1-2 septatis, 18 μ -25 μ longis, 3 μ -5 μ latis.

In foliis *Hicoriae pecanae*.

Perithecia hypophyllous, flask-shaped, single or in groups of twos or threes; usually found scattered among the older pycnidia; from 300 μ to 350 μ wide and 150 μ to 250 μ high without the beaks. Beaks from 200 μ to 250 μ long by 50 μ to 60 μ wide. Walls of perithecia 2 cells thick at bottom and top and 3 to 4 cells thick on sides; dark brown. Asci hyaline, 8-spored with pore at apical end, cylindrical, thin-walled, 45 μ to 50 μ by 6 μ to 8 μ . Ascospores 22 μ to 28 μ by 3 μ to 4 μ , straight or curved, hyaline, guttulate, and 1-septate with setae at both ends.

Pycnidia superficial, subglobose, reddish brown, 75 μ to 300 μ in diameter. Conidia 15 μ to 20 μ by 2 μ to 3.5 μ , continuous, hyaline, curved, fusoid, and attenuated toward the apex. Conidiophores simple, hyaline, 18 μ to 25 μ by 3 μ to 5 μ and 1- to 2-septate.

On leaves of *Hicoria pecan*.

Type specimens have been deposited in the Pathological Collections of the Bureau of Plant Industry, United States Department of Agriculture, at Washington; in the New York Botanical Garden; and in the Missouri Botanical Garden.

GROWTH ON ARTIFICIAL MEDIA

The liver-spot fungus was easily cultured by streaking corn-meal agar plates with either conidia or ascospores. Monospore cultures became macroscopic in about 10 days. From the beginning, the mycelium was very compact, light tan in color, and covered with short aerial hyphae. Production of conidia began in about 20 days, at the optimum temperature. The conidia were borne in pycnidia having the same characteristics as those on the host.

The fungus grows on a wide variety of artificial media. The best growth was made on corn-meal agar, when an area 10 cm in diameter was covered in 60 days. It fruited best on a combination of corn-meal and pecan or corn-meal and hickory agar, made by rolling pecan or hickory leaves and placing them in test tubes of corn-meal agar. In 10 days a few pycnidia appeared, and after 4 weeks the entire surface of the medium was covered with the fruiting bodies. On vegetable plugs, especially potato, an abundance of pinkish aerial hyphae was produced, but no conidia were present.

¹² WOLF, F. A. (See footnote 8.)

When kept in an incubator for 60 days on corn-meal and potato agar, the fungus grew best at a temperature of 24° C. At this temperature the maximum growth was 5½ cm. The mycelium was hyaline to light tan and mostly submerged, with little aerial hyphae. Little growth was made at 28° and none at 34° C. At 16° conidia and an abundance of white aerial hyphae were produced, although the growth was only 2 cm in diameter. No growth occurred at 12°.

During the course of hydrogen-ion studies, corn-meal agar medium with a pH range of from 4.0 to 8.5 was used. The optimum growth, an area 4 cm in diameter occurred between pH 5.8 and pH 7.0, whereas poor growth was made at pH 4.0 and pH 8.5. The general characteristics of the growth were the same at all of these hydrogen-ion concentrations. The mycelium was light brown, mostly submerged, with little or no aerial hyphae. Where growth was optimum the medium was discolored, changing from hyaline to light brown. Conidia were produced in all the cultures.

PATHOGENICITY

During the season of 1930 unsuccessful attempts were repeatedly made to produce liver spot by using ascospores from leaves as inocula. In 1931 all inoculations were made with conidia from leaves collected during the late fall of 1930 and from ascospore cultures. The imperfect and perfect stages of the fungus had previously been connected when perithecia of *Gnomonia* were found among the old pycnidia of the *Leptothyrium* stage and conidia were produced in cultures made from monospore cultures of the ascigerous stage. A mass of conidia was removed from culture tubes and from leaves by means of a scalpel and placed on both the dorsal and ventral surfaces of leaves of the Stuart, Van Deman, and Pubst varieties. The leaves were then enclosed in glassine bags for 48 hours. These inoculations were begun the 1st of May, while the leaves were young and before they had taken on a dark-green color. The inoculations were made in series every 2 weeks throughout the summer. The first infections were recorded September 9 on leaves that were inoculated July 28 with conidia taken from leaves and ascospore cultures. The results of inoculations on young and on older leaves were practically the same, although the percentage of infection was small. The later the inoculations were made, the higher was the percentage of infection; maximum infection occurred in October from inoculations made in September. The results with *Gnomonia* from the Stuart variety of pecan and the shagbark hickory (*Hicoria ovata*) were as follows: 58-percent infection was obtained on pecan with conidia from the hickory *Gnomonia* and 63 percent on hickory with conidia from the pecan *Gnomonia*, whereas 90-percent infection was obtained on pecan with conidia from the pecan *Gnomonia*, and 97 percent on hickory with conidia from the hickory *Gnomonia*. There was 48 percent *Gnomonia* infection on the pecan checks and 55 percent on the hickory checks. All infections were on the lower or dorsal surface of the leaves, indicating that the fungus enters the host through the stomata, since most of the stomata are on the lower surface. The period of incubation for infection was from 5 to 6 weeks for both old and young leaves,

indicating that the age of the leaves is an unimportant factor in the susceptibility to the liver-spot pathogene.

Temperature and humidity were doubtless the chief factors repressing infection earlier in the season, for natural infections of liver spot were present in the field during June and July.

VARIETAL SUSCEPTIBILITY

While most of the improved varieties of pecan are attacked by the liver-spot fungus, some are more susceptible than others. Among the most susceptible are the Stuart, Van Deman, and Pabst. In the vicinity of Shreveport, La., the Schley and Georgia Giant are highly resistant. Most of the native pecans in Texas are attacked, some being defoliated.

CONTROL

During the season of 1930, experiments were conducted on the Pabst variety in an effort to control the liver-spot disease. A block of trees was sprayed with one application of 4:6:50 bordeaux mixture on June 23. As the spring of 1930 was very early, most of the ascospores were discharged by May 1. The spraying was too late, for more than 50 percent of infection occurred. The experiment was repeated in 1931 on the Stuart variety, the application of 4:6:50 bordeaux mixture being made on May 27. The spring was later and the ascospores were not discharged until about May 10. There was not more than 2- to 3-percent infection from the liver-spot fungus on the sprayed trees, as compared with 75-percent infection on the unsprayed trees. The unsprayed trees were defoliated by September 1, whereas the sprayed trees held their foliage until December. These experiments indicate that in the vicinity of Shreveport, La., the fungus would be comparatively easy to control if the trees were sprayed during the latter part of May.

Sanitation may be an important factor in controlling the disease. If the leaves were destroyed by burning or if they were plowed under deeply enough in the fall or early spring, the early infection would undoubtedly be greatly reduced.

SUMMARY

Liver spot, a foliage disease of pecans, caused by *Gnomonia caryae* var. *pecanae*, nov. var., has been collected in Mississippi, Arkansas, Louisiana, and Texas.

The disease first appears on the lower surface of the leaves as circular brown dots resembling liver in color. Since the name "western sooty spot" now in use indicates that the leaf spot is black, which is not the case, the writer believes that "liver spot" would be a better term for this disease.

The fungus causes the greatest injury to trees of low vitality, but it also attacks vigorous trees and occasionally causes premature defoliation. It seems to thrive best in climates of high temperature and low rainfall.

The conidial stage of the fungus is considered as *Leptothyrium caryae* var. *pecanae*, distinct from *L. nervisedum* Cole. The perfect stage is considered as *Gnomonia caryae* var. *pecanae*, distinct from

G. setacea var. *macrospora* Ell. and Ev., from a 2-spored *Gnomonia* described by Matz in Florida, and from *G. caryae* Wolf.

The liver-spot fungus will grow on a variety of laboratory media, but fruits best on a combination medium of pecan or hickory leaves rolled and inserted in a test tube containing corn-meal agar. The optimum temperature for growth is 24° C. and the minimum is between 12° and 16°.

The writer was able to prove, according to Koch's rules, that the liver-spot disease is caused by *Gnomonia caryae* var. *pecanae*.

Varietal resistance was noted among pecans. The varieties Schley and Georgia Giant were highly resistant, whereas the Stuart, Van Deman, and Pabst varieties were very susceptible.

One application of 4:6:50 bordeaux mixture on May 27, 1931, gave almost perfect control of the liver-spot fungus.

FLOODING TO CONTROL ROOT-KNOT NEMATODES¹

By LLOYD N. BROWN²

Agent, irrigation assistant, Division of Irrigation, Bureau of Agricultural Engineering, United States Department of Agriculture

INTRODUCTION

Use-of-water studies in the Sacramento-San Joaquin Delta were undertaken in 1924, and are still being continued. The delta, about 400,000 acres in area, is composed of peat and sedimentary soil, the latter type amounting to about 60 percent of the whole. The channels of the delta connect with an upper arm of San Francisco Bay and are subject to tidal action. This area, formerly a tidal swamp, was reclaimed by building levees around sections varying in size from a few hundred to several thousand acres. The ground surface of most of the enclosed areas is several feet below low-tide level. Irrigation water is withdrawn from the delta channels by siphoning it over the levees or carrying it under them in culverts, and is distributed to the land through a system of ditches. Excess water is collected in drainage canals and returned to the channels by pumping.

Work on the consumptive use of water, referred to above, consisted in part of growing crops on peat and sedimentary soil in typical moisture-requirement tanks. While this work was in progress, considerable difficulty was experienced with the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. The damage caused by the nematodes in the delta is of considerable magnitude. Great areas of very valuable land are rendered unfit for the profitable production of potatoes, sugar beets, and other crops. No effective system of control is known. The nematode population can be reduced by replacing susceptible crops with a rotation of nonsusceptible crops such as barley or corn, or by burning off the surface layer of the peat, which is a very undesirable practice. Flooding had been suggested, but results obtained were neither conclusive nor in agreement. The method, however, appeared to offer possibilities and was definitely related to use-of-water studies.

¹ Received for publication Sept. 28, 1933, issued February 1934. The nematode control experiments reported herein, made in the period 1928-30, were incident to work on the consumptive use of water in the Sacramento-San Joaquin Delta, Calif. The project as a whole was carried on under a cooperative agreement subscribed to by the following parties: U. S. Department of Agriculture, Bureau of Public Roads, operating through the Division of Agricultural Engineering; State of California, Department of Public Works, operating through the Division of Engineering and Irrigation and the Division of Water Rights; University of California, Agricultural Experiment Station, operating through the Division of Irrigation Investigations and Practice. Reorganization has now (1933) resulted in the Federal agency becoming the Bureau of Agricultural Engineering, operating through its Division of Irrigation, and the State agency becoming the Division of Water Resources in the Department of Public Works.

² Gerald Thorne, Division of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture, was detailed to advise and assist in planning the experiment. His services included two visits to King Island and microscopic examinations of soil and water samples. Jocelyn Tyler, Division of Entomology and Parasitology, University of California, did all greenhouse work and gave advice on numerous occasions. The California Delta Farms, Inc., through George Atherton, general manager, provided the site and water supply for the experiment and in other ways facilitated the installation. O. V. P. Stout, irrigation engineer, Bureau of Agricultural Engineering, who supervised the consumptive use of water studies, encouraged and forwarded the work from its inception to completion.

The larvae of *Heterodera marioni* are very slender, averaging 0.4 mm in length, and even on a dark background are barely visible to the unaided eye. Three conditions are necessary for their optimum

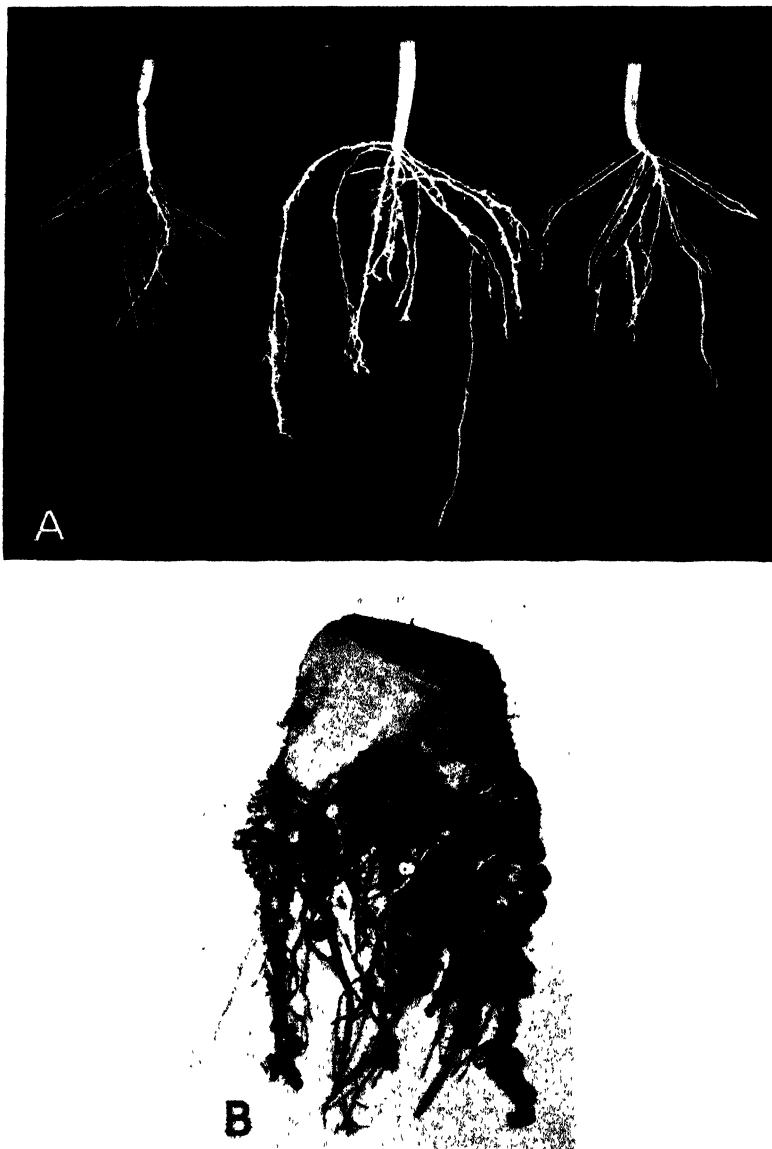


FIGURE 1.—A, Nematode-infested roots of pumpkin; B, nematode-infested roots of sugar beet.

growth—proper temperature, moisture, and air. Tyler³ found that activity begins at about 55° F. and increases up to about 80°, after which it falls off rapidly. They multiply most rapidly when the sur-

³ TYLER, J. DEVELOPMENT OF THE ROOT-KNOT NEMATODE AS AFFECTED BY TEMPERATURE. *Hilgardia* 7: 381-415, illus. 1933.

rounding soil is of proper moisture content for plant growth. The greatest numbers are found in the upper foot of soil, which is best aerated, although some may be found at a depth of several feet. In other words, they do best under conditions that are most favorable for the growth of plant roots. Some of the most common commercial plants subject to attack are potato, sugar beet, celery, tomato, melon, beans, cantaloupe, squash, cucumbers, peaches, and figs. Two views of roots infested with the nematodes are shown in figure 1.

REVIEW OF LITERATURE

Several writers have mentioned the possibility of controlling nematodes by flooding, but no precise work appears to have been done. Bessey,⁴ after discussing the problem, sums up:

Flooding, then cannot be recommended as a certain means of exterminating root-knot under all circumstances. Probably the soil should be flooded at least 25 days; in the laboratory the nematode larvae usually succumbed much sooner when isolated and placed in water.

Frandsen,⁵ in reporting some flooding experiments, states:

It has been suggested that fields might be rid of the worms by flooding them with water but our experiments indicate that at least 3 months submersion would be necessary to make this method effective.

Watson,⁶ who has made some observations on flooding as a means of controlling root-knot, concludes:

Nematodes can be greatly reduced in number, if not completely eradicated, by flooding. Indeed, in some fields usually flooded during part of the summer, as with tomato fields on the east coast, nematodes are seldom noticed. There is yet much to be learned as to the length of time a field should be covered with water, especially at different seasons, and as to the resistance of the eggs to water. Generally a week or two is considered necessary.

No flooding experiments were continued long enough to determine the time necessary to secure complete control.

It may be noted here that nematode larvae are not drowned by submergence in water, but supposedly succumb to exhaustion induced by their own activity, which is increased in water.

INSTALLATION OF PLOTS

A site, possessing the three prime requisites—unfailing water supply, adequate drainage, and heavy nematode infestation—was selected on King Island, Calif., in April 1928. King Island, a tract of about 3,300 acres, lies with its southeast corner about 7 miles west and 6 miles north of Stockton. Two microscopic examinations of the peat soil showed populations of *Heterodera marioni* estimated at 8 and 45 millions per acre. A crop of potatoes planted on the area 3 or 4 years previously had been a failure because of nematodes. Barley was grown on the area in 1926 and 1927. On May 8, 1928, barley about 2 feet high was cleared from the plot area. Many volunteer potatoes heavily invested with nematodes were found growing with the barley.

Twenty plots, each 12 feet square, arranged in a double row 10 plots long from east to west, or, in other words, 10 pairs of plots, were prepared. Each plot was enclosed by a watertight wall or curb made of tongue-and-groove redwood, extending 12 inches above and 18 inches below the ground surface. The tops of all the curbs were at the same

⁴ BESSEY, E. A. ROOT-KNOT AND ITS CONTROL. U.S. Dept. Agr. Bur. Plant Indus. Bull. 217. 82 pp. illus. 1911.

⁵ FRANDSEN, P. EELWORM PARASITES OF PLANTS. Calif. Conn. Hort. Mo. Bull. 5: 60-62. 1916

⁶ WATSON, J. R. CONTROL OF ROOT-KNOT, II. Fla. Agr. Expt. Sta. Bull. 159, pp. 29-44. 1921.

level throughout. Two views of the plots are shown in figure 2. The water supply was obtained from the channel nearby through 5-inch slip-joint pipe. The pipe discharged into a delivery box at the northeast corner of the plots. The north side of this box was built about 6 inches lower than the tops of the curbs enclosing the plots, thus providing a spillway which protected the plots from overflowing. The west end of the delivery box was connected to a wooden flume 10 inches deep and 12 inches wide built on the north side of the plots. Water was admitted to each pair of plots through holes in the north curb of the north plot of the pair, which was also the south side of the flume, and thence through auger holes in the middle curb to the south plot of the pair. With this set-up it was possible to hold water on all or any pair or pairs of the plots.

In addition to the natural infestation of nematodes, some badly affected roots of smartweed, *Polygonum acre* H.B.K., were buried a few inches deep at marked spots in each plot. This precaution was taken in order to have a small sample of soil for microscopic examination which was definitely known to contain nematodes.

After several trial floodings, flooding was continuous for the respective periods in each case, from July 18, 1928, until the end of the experiment, with the exception of a few unavoidable interruptions, none of which was for more than 24 hours' duration and most of them for considerably less.

FLOODING EXPERIMENTS ON KING ISLAND PLOTS

In preliminary experiments, peat soil infested with nematodes was submerged in small tanks. Several microscopic examinations of the soil up to and including one 3 months after flooding showed many living larvae. However, examination on the fourth month after flooding revealed only apparently exhausted larvae.

On November 29, 1928, slightly more than 4 months after flooding, samples of soil from the plots containing *Polygonum acre* roots were examined microscopically. No nematodes or eggs were found.

On January 22, 1929, after slightly more than 6 months of flooding, another similar set of samples was taken and examined microscopically. This examination also failed to disclose any nematodes or eggs.

On the same date two 6-inch and twelve 4-inch pots were filled with flooded soil and a tomato plant was placed in each. On March 1, 1929, after the plants had been in the greenhouse about 5 weeks, 9 galls were found on the roots in one 6-inch pot and 1 each in 4 of the 4-inch pots. The other plants appeared to be free from nematodes.

When the above-noted samples were taken, 6 plots were drained. On June 1, about 5 months after draining, these plots were planted with pumpkin seed. On July 5, 35 days later, the pumpkin roots showed many galls, microscopically determined to be caused by nematodes. Other plants growing in the plots, and susceptible to nematode attack, also showed galls.

From the foregoing observations it would appear that from 4 to 6 months' submergence will kill nematode larvae, but viable eggs remain and under favorable conditions these will hatch.

On July 18, 1929, 6 more plots were drained after having been under water for 12 months. On August 9 all plots were planted with pumpkin seed. Soil and pumpkin-root samples taken September 12 failed to show any nematodes when examined microscopically. However,

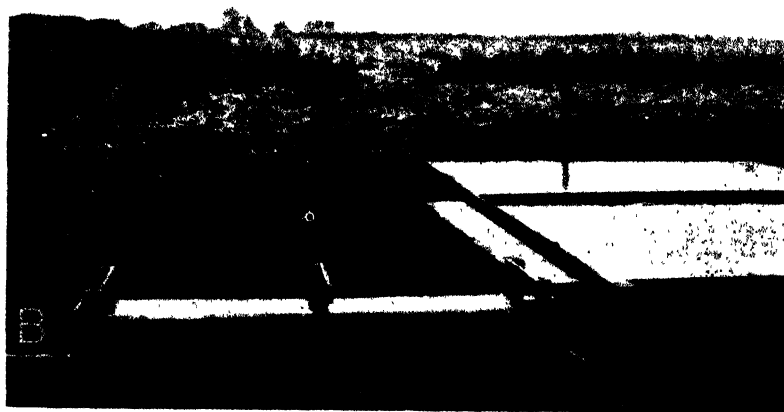
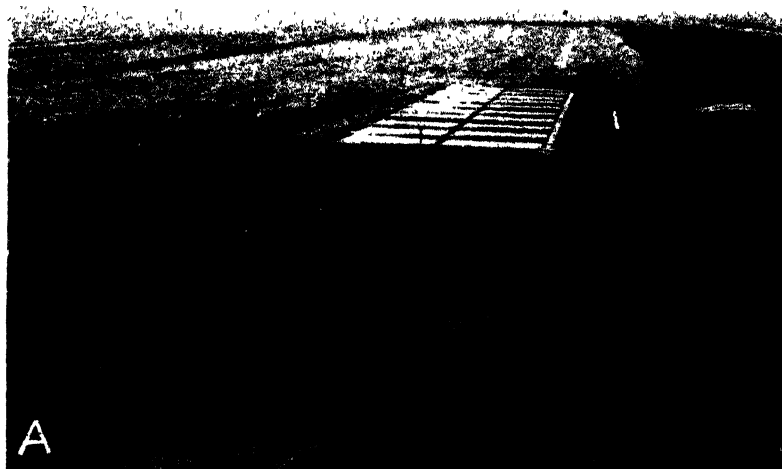


FIGURE 2.—*A*, General view of nematode plots on King Island, west from top of levee. Note drainage ditches. *B*, East end of nematode plots on King Island, showing supply pipe, receiving box, spillway, flume, and plots nos 1 and 2.

when pumpkin roots were dug out on October 9 some nematode galls were found. Hence, it would appear that after a year's flooding some of the nematodes had lived over in egg form, although apparently in greatly reduced numbers.

On June 4, 1930, 22½ months after flooding, 4 more plots were drained, and on June 18 these were planted to pumpkins. Roots of pumpkins were examined on July 10 and 26 and again in October, but in no case were any nematode galls found. *Polygonum acre* grew in the plots, but no evidence of nematodes was observed on the roots. Therefore it appears that all viable nematodes had hatched and the larvae died during 22½ months of flooding. Apparently between 12 and 22½ months of flooding was necessary to rid the land of nematodes. Under ordinary conditions this would probably be considered too long a period to be of practical value. However, under conditions similar to those prevailing in the Sacramento-San Joaquin Delta it may be practicable to flood for 2 years in order to rid the land of this pest, especially when prices of farm produce are low.

FLOODING EXPERIMENTS ON EMPIRE TRACT

The Empire tract, an island of about 3,000 acres of peat soil, lying just west of King Island, was flooded from about May 22 to September 22, 1929, in an effort to leach out alkali, kill weeds, and improve the soil. Previous to the flooding, four samples of soil badly infested with nematodes were obtained by pressing 9½-inch lengths of slipjoint pipe into the soil and then digging them out, thus removing a pipe full of soil. Two samples contained small sugar beets, the other contained *Polygonum acre* plants. The filled pipes were then buried with the upper ends even with the soil surface on the island to be flooded.

The pipes of soil were dug up after the 4 months of flooding. A microscopic examination of the material from 2 of the cylinders, 1 each of the sugar beet and *Polygonum acre* roots, failed to show any nematodes. The soil from the other 2 cylinders was placed in eighteen 4-inch flowerpots in a greenhouse February 11, 1930. On March 25 a *P. acre* plant in one pot had 5 galls, a *P. acre* plant in another pot had 2 galls, and a tomato plant had 4; the tomato plants in the other pots had respectively, 4, 4, 5, 6, 7, 9, 9, 9, 9, 10, 10, 13, 13, 17, 25, and 34 galls. These results are consistent with those obtained on the King Island plots, in indicating that 4 months' flooding will kill larvae but that viable eggs still remain.

SUMMARY AND CONCLUSIONS

Four months' submergence of soil containing the nematode *Heterodera marioni* killed the larvae, but the eggs remained viable. Flooding for 6 months gave approximately the same results as for 4 months. Nematodes appeared in soil flooded for a year, although apparently in greatly reduced numbers. After soil had been submerged for 22½ months no nematodes were found when plants were grown in it.

Therefore, it may be concluded that about 4 months' of submergence kills *Heterodera marioni* larvae, but the eggs are not killed until sometime between the end of the twelfth month and midway of the twenty-third month; hence, to rid nematode-infested soil by continuous flooding would entail the loss of 2 years' crops.

CYSTINE DEFICIENCY OF SOYBEAN PROTEIN AT VARIOUS LEVELS, IN A PURIFIED RATION AND AS A SUPPLEMENT TO CORN¹

By CHARLES L. SHREWSBURY, *associate chemist*, and JOHN W. BRATZLER,² *senior student, Purdue University Agricultural Experiment Station and College of Agriculture*

INTRODUCTION

The extensive use of soybeans (*Soja max*) and soybean products in livestock rations, as well as in the dietary of man, makes information on the nutritive properties of this legume of importance. Mitchell and Smuts³ recently published data which strongly indicate that a ration containing 10 percent of soybean protein (ether extracted soybeans) is deficient in cystine. The experimental period was short (5 weeks), and the rations were not readily consumed, resulting in very limited growth.

Csonka and Jones⁴ have recently analyzed the glycinin from 10 varieties of soybeans for cystine and other amino acids. The glycinin from Manchu soybeans contained next to the highest amount of cystine (1.45 percent).

Soybeans are generally fed to livestock as a supplement to corn or other carbohydrate-rich feed. The experiments described in this paper were designed to reinvestigate the reported deficiency of soybean protein at a level of 10 percent, to determine whether a cystine deficiency existed at a protein level of 15 percent, and whether a ration made from corn and soybeans, such as is used in swine feeding, would exhibit a cystine deficiency.

EXPERIMENTAL PROCEDURE

Two methods of feeding were used in the experiments: In one series feed consumption was equalized; in the other the animals (rats) were allowed to eat ad libitum. In both series the animals were separated into pairs, one animal of a pair receiving the control ration and the other the cystine-supplemented ration. The animals of a pair were selected from stock shortly after weaning and were of the same age and sex, similar in weight, and from the same litter. Thus, the only difference in the two series was that in one case feed consumption was equalized, whereas in the other feed was available at all times. It was felt this would give some information as to the relative merits of the two methods of experimentation. The experimental period was 8 weeks.

The yellow corn and the Manchu variety of soybeans used in the experiments were obtained from the experimental swine farm and were of good quality. The soybeans were extracted with ether for several

¹ Received for publication Sept. 28, 1933; issued February 1934.

² A part of the material in this paper was submitted by the junior author to the School of Agriculture, Purdue University, as a thesis in partial fulfillment of the requirements for the bachelor of science degree.

³ MITCHELL, H. H., and SMUTS, D. B. AMINO ACID DEFICIENCIES OF BEEF, CORN, OATS, AND SOYBEANS FOR GROWTH IN THE WHITE RAT. *Jour. Biol. Chem.* 95: 263-281. 1932.

⁴ CSONKA, F. A., and JONES, D. B. DIFFERENCES IN THE AMINO ACID CONTENT OF THE CHIEF PROTEIN (GLYCININ) FROM SEEDS OF SEVERAL VARIETIES OF SOYBEAN. *Jour. Agr. Research* 46: 51-55 1933

days and dried. The yellow corn and ether-extracted soybeans had a crude protein content of 9.5 and 51.7 percent, respectively. The other materials used in the rations were of good quality. Table 1 gives the composition of the rations.

TABLE 1. *Composition of rations*

Constituent	Percentage composition of ration no —					
	261	262	261	265	259	260
Ether-extracted soybeans	11.5	11.5	29.0	29.0	20.0	20.0
Yellow corn	84.0	84.0				
Dextrin	2.5	2.25	54.0	53.75	63.0	62.75
Butter			8.0	8.0	8.0	8.0
Yeast			2.0	2.0	2.0	2.0
Cod-liver oil			2.0	2.0	2.0	2.0
Salts ^a	2.0	2.0				
Osborne-Mendel salts ^b			4.0	4.0	4.0	4.0
NaCl			1.0	1.0	1.0	1.0
Cystine		25		25		25
Crude protein from soybeans	5.94	5.94	14.99	14.99	10.34	10.34

^a Limestone 10 parts, special steamed bone meal 10 parts, common salt 1 part

^b OSBORNE, T. B., and MENDEL, L. B. NUTRITIVE VALUE OF THE WHEAT KERNEL AND ITS MILLING PRODUCTS. Jour Biol Chem 37: 572 1919

The cystine used in the experiments was obtained from the Eastman Kodak Co. and was labeled L-cystine. It was found to be 97.25 percent pure, based on its nitrogen analysis.

EXPERIMENTAL RESULTS

The supplementing effect of cystine on a purified ration containing 10 percent of soybean protein is seen by an examination of table 2.

TABLE 2.—*Supplementing effect of cystine on a purified ration (259) containing 10 percent of soybean protein when fed to rats, as indicated by their weights in grams*

[Experimental period 56 days]

FEED CONSUMPTION EQUALIZED

Item	Pair 1		Pair 2		Pair 3		Pair 4	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	43	40	42	40	46	44	49	47
Final weight	71	71	59	64	86	102	78	91
Total gain	28	31	17	24	40	58	29	44
Total feed	208	208	241	241	282	283	201	201

FEED CONSUMPTION UNRESTRICTED

Initial weight	38	36	34	34	30	29	35	33
Final weight	59	52	47	58	56	55	46	74
Total gain	21	16	13	24	26	26	11	41
Total feed	216	218	114	118	135	123	122	180

* Died before the end of the experiment, pair mates receiving cystine were continued on and were all alive at the end of the experiment.

TABLE 2.—*Supplementing effect of cystine on a purified ration (259) containing 10 percent of soybean protein when fed to rats, as indicated by their weights in organs—Continued*

Item	Pair 5		Pair 6		Pair 7		Pair 8	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	42	40	39	38	47	47	45	43
Final weight	59	64	^a 69	74	^a 97	86	^a 67	75
Total gain	17	24	30	36	28	39	22	32
Total feed	211	241	228	228	236	236	168	169

FEED CONSUMPTION UNRESTRICTED								
Initial weight	30	29	36	35	35	33	46	46
Final weight	^a 40	65	^a 45	51	^a 57	59	^a 74	93
Total gain	10	36	9	16	22	26	28	47
Total feed	117	147	157	235	127	111	197	201

^a Died before the end of the experiment, pair mates receiving cystine were continued on and were all alive at the end of the experiment.

The results of all the feeding trials have been summarized statistically according to the method of Student ⁵ in table 3. In the experiments in which feed consumption was equalized all of the pair mates receiving cystine in the ration made larger gains than the controls. Although the rations were consumed in considerable quantity, growth was relatively poor. Five of the control animals died before the end of 50 days, while none of the pair mates receiving cystine had succumbed by the end of the experiment. The weights and feed consumptions recorded in the table for the pair mates that died are for the last period preceding death. A marked difference was noted between the hair coats of the rats; the ones receiving cystine were smooth and well kept, while the controls had rough coats.

TABLE 3. *Statistical results of experiments with cystine as a supplement in the diet of rats*

Item	Soybean protein 10 percent (ration no 259), feed equalized	Soybean protein 10 percent (ration no 259), feed unrestricted	Soybean protein 15 percent (ration no 264), feed equalized	Soybean protein 15 percent (ration no 264), feed unrestricted	Corn and soybean protein (ration no 261), feed equalized	Corn and soybean protein (ration no 261), feed unrestricted
Total mean difference in gain = M	^a +9.6	^a +11.1	^a +12.1	^a +10.5	^a +0.17	^b -9.9
Standard deviation S	4.95	12.5	6.31	14.1	6.03	21.4
Ratio $M/S = V$	1.97	.92	1.93	.77	.028	.46
Probability P	.0006	.026	.0007	.041		.1332
Probable error	± 1.17	± 3.94	± 1.48	± 3.32	$\pm .943$	± 5.0
Observation number	8	8	8	8	18	8

^a + = Total mean difference in total gain in weight favors cystine-supplemented ration

^b - = Total mean difference in total gain in weight favors control ration

The data obtained from the experiments in which the animals had unrestricted access to the feed cannot be said to establish definitely the cystine deficiency of soybean protein. Five pair mates receiving cystine made better gains than did the controls, two made about the same gain, and one did not gain so much. The mortality among the control animals was pronounced. Feed records in the case of rations

of this type may, perhaps, be open to some question, since losses due to wastage are unavoidable.

The statistical treatment of the data reveals striking differences in the accuracy of the two feeding methods. The probability that the difference in gain in favor of the cystine-supplemented ration was due to chance was very small in the experiments in which feed consumption was equalized. The probable error of the result ± 1.17 is also quite small. (Probable error is in terms of grams of gain). However in the experiments in which access to feed was unrestricted the probability that the results were due to chance was larger ($P = 0.026$). The probable error was also larger, ± 3.94 . It would seem that where unequal feed consumption might produce irregular gains a larger number of experiments would be required to establish a given result.

The relatively poor growth and high mortality obtained by feeding the purified ration containing 10 percent of soybean protein led to an investigation of the possible cystine deficiency of soybean protein fed at a level of 15 percent. This is nearer the amount of protein that is considered to be necessary for satisfactory growth of rats. The results of this work are presented in table 4.

TABLE 4.—*Supplementing effect of cystine on a purified ration (264) containing 15 percent of soybean protein when fed to rats, as indicated by their weights, in grams*

[Experimental period 56 days]

FEED CONSUMPTION EQUALIZED

Item	Pair 1		Pair 2		Pair 3		Pair 4	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	45	44	43	41	42	41	35	35
Final weight	97	104	90	98	91	110	* 66	77
Total gain	52	60	47	57	49	69	31	42
Total feed	453	453	401	401	390	390	169	169

FEED CONSUMPTION UNRESTRICTED

Initial weight	38	38	42	39	37	37	37	37
Final weight	103	109	96	135	93	99	* 74	65
Total gain	65	71	54	96	56	62	37	28
Total feed	405	355	407	442	327	347	220	179

FEED CONSUMPTION EQUALIZED

Item	Pair 5		Pair 6		Pair 7		Pair 8	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	39	36	41	39	42	41	41	40
Final weight	81	85	92	114	92	100	* 82	89
Total gain	42	49	51	75	50	59	41	49
Total feed	372	372	420	420	423	423	231	231

FEED CONSUMPTION UNRESTRICTED

Initial weight	37	37	48	46	39	38	37	35
Final weight	* 90	85	* 93	105	95	121	69	89
Total gain	53	48	45	59	56	83	32	71
Total feed	219	178	176	204	388	450	217	194

* Died before the end of the experiment; pair mates receiving cystine were continued on that diet and were all alive at the end of the experiment.

Although feed consumption was higher and growth was greater than when 10 percent of soybean protein was fed, a definite increase in rate of gain was obtained when cystine was included in the ration. The mortality was reduced when the level of soybean protein amounted to 15 percent of the ration. All the deaths that occurred were of animals on the unsupplemented control ration. The quality of the hair coat was better in the animals receiving the cystine supplement, as was the case when 10 percent of soybean protein was fed.

The supplementing effect of cystine on soybean protein could be deduced, perhaps, from the experiments in which the animals had unrestricted access to feed, since all but two of the pair mates receiving cystine made larger gains than the controls. In 6 of the 8 pairs of rats that had access to food at all times, the rat that consumed the most feed made the better gain.

A consideration of the statistical results demonstrates that the experiments in which feed consumption was equalized gave the most reliable results. The probable error of the mean result was higher and the probability that the result was due to chance was also higher in the series of experiments, in which unrestricted access to feed was allowed.

The possible significance of the cystine deficiency of soybeans in the feeding of livestock is interesting. It has been demonstrated⁶ that a ration prepared from corn, soybeans, and minerals, in which soybeans furnish approximately 6 percent of protein, is not adequate for satisfactory growth of rats and swine. Cooking or roasting the soybeans in this ration results in a definite improvement in its growth properties. Since soybeans or soybean products are utilized in practice as a protein supplement to corn or other carbohydrate feed, it seemed worth while to determine whether cystine would supplement a ration likely to be used in farm practice. The results might help to explain the change that soybeans undergo during cooking or roasting. The results given in table 5 show that a cystine supplement does not improve the ration in which yellow corn is present.

⁶ SHREWSBURY, C. L., VESTAL, C. M., and HAUGR, S. M. THE EFFECT OF YEAST AND CANKIN SUPPLEMENTS TO CORN AND SOYBEAN RATIONS WHEN FED TO RATS AND SWINE. *Jour. Agr. Research* 44 267-274, illus 1932.

TABLE 5.—*Supplementing effect of cystine on a ration (261) of yellow corn and 5.94 percent of soybean protein when fed to rats, as indicated by their weights in grams*

[Experimental period 56 days]

FEED CONSUMPTION EQUALIZED

Item	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	37	35	46	46	47	47	47	46	44	43	37	38
Final weight	95	89	129	137	113	119	122	130	114	115	105	97
Total gain	58	54	83	91	66	72	75	84	70	72	68	59
Total feed	384	384	553	553	466	466	469	469	467	467	405	405

Item	Pair 7		Pair 8		Pair 9		Pair 10		Pair 11		Pair 12	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	36	36	39	40	41	41	38	37	52	50	39	36
Final weight	107	99	117	115	155	163	119	122	146	139	95	97
Total gain	71	63	78	75	114	122	81	85	91	89	56	61
Total feed	432	432	466	466	626	626	470	468	534	533	384	385

Item	Pair 13		Pair 14		Pair 15		Pair 16		Pair 17		Pair 18	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	37	36	36	38	34	31	45	45	39	40	37	40
Final weight	125	124	117	114	116	114	122	116	124	131	123	120
Total gain	88	88	81	76	82	83	77	71	85	91	86	80
Total feed	501	503	478	475	442	447	506	501	501	502	489	487

FEED CONSUMPTION UNRESTRICTED

Item	Pair 1		Pair 2		Pair 3		Pair 4	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	52	52	36	35	35	35	47	47
Final weight	165	177	157	107	124	106	121	101
Total gain	113	125	121	72	89	71	74	54
Total feed	700	669	582	322	452	378	455	328

Item	Pair 5		Pair 6		Pair 7		Pair 8	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	49	51	52	53	35	35	34	35
Final weight	151	163	141	146	132	138	122	99
Total gain	102	112	89	93	97	103	88	64
Total feed	669	680	598	547	535	455	489	295

* Dead at end of 42 days; pair mate receiving cystine alive at end of 53 days.

In both series of experiments only 50 percent of the pair mates receiving cystine made larger gains than the controls. In the experiments in which feed consumption was equalized it was not possible to determine the degree of probability that the small difference in

growth in favor of the cystine-supplemented ration was due to chance, since Student's tables do not furnish probability figures for values of Z lower than 0.1. However, an inspection of the value of 0.1 ($P=0.3425$) shows that it is unlikely that the mean increase in gain in favor of the cystine supplemented ration is significant.

SUMMARY

The possible cystine deficiency of soybean protein fed at levels of 10 and 15 percent in a purified ration, and at a level of 6 percent as a supplement to corn, was studied by means of the paired-feeding method in which feed consumption was equalized and in similar experiments in which the animals (rats) had unrestricted access to feed.

Soybean protein (ether-extracted soybeans) was found to be definitely deficient in the amino acid cystine when the protein was fed at a level of 10 or 15 percent in a purified ration.

A ration made from corn, soybean protein, and minerals, such as is used in livestock rations, was not improved by the addition of L-cystine.

The advantages of the paired-feeding method in which feed consumption is equalized, in the determination of amino acid deficiency by means of nutrition experiments with rats, is demonstrated by these experiments, as compared with unrestricted consumption.

PLUMAGE AND EYE COLOR INHERITANCE IN THE SINGLE COMB RHODE ISLAND RED FOWL¹

By D. C. WARREN, *professor of poultry husbandry*, and C. D. GORDON, *graduate assistant, Kansas Agricultural Experiment Station*

STANDARD COLOR OF BREED

An examination of a flock of Rhode Island Red fowls shows the color of the plumage and eyes to be highly variable. In the average flock there are birds varying from a light shade (approaching buff) to a deep mahogany or bay color. The eyes also show a wide variation in the amount of red pigment in the iris. These variations exist in spite of the fact that the breed has been selected for years for a deep red color of both plumage and iris. The wide range of intensity of pigmentation found in this breed would seem to suggest that the ideal standard color is rather complex in the nature of its inheritance.

The American Standard of Perfection² gives the description of the ideal bird of the Rhode Island Red breed as specified by the American Poultry Association. This standard requires that the Rhode Island Red be of a deep reddish bay color throughout the plumage of both sexes. It is desired that all sections of the plumage be as uniform in color as possible. The under fluff of the feathers should also be of the same color as the surface.

Tipping of black is permitted in the neck feathers of females. Black color is also desired in the primary and secondary flight feathers of both sexes. The upper web of the primary feathers should be red and the lower web largely black. In the secondaries the color is reversed, the lower web being red and the upper one largely black. The main tail feathers of both male and female are black. The sickle and covert feathers are also black or greenish black in the males. The occurrence of white in any section of the plumage and of black, except where specified, constitutes a defect in color.

Very little has been published on the inheritance of the color qualities of the Rhode Island Red. Bittenbender³ recommended that both male and female be the same color, and he further mentioned the necessity of having a bar of black pigment in the back feathers of only one parent to assure rich, red plumage color in the offspring.

Dunn⁴ suggested that the Rhode Island Red carries the recessive gene e^m which restricts melanic pigment to the neck, wings, and tail. The standard established for this breed has caused the melanic pigment to be reduced to a minimum in regions, except the flight and tail feathers.

¹ Received for publication July 24, 1933; issued February 1934. Contribution No. 76 from the Department of Poultry Husbandry, Kansas Agricultural Experiment Station.

² AMERICAN POULTRY ASSOCIATION. THE AMERICAN STANDARD OF PERFECTION. . 1930 ed., 487 p., illus. n.p. 1930

³ BITTENBENDER, H. A. BREEDING RHODE ISLAND REDS FOR STANDARD TYPE AND EGG PRODUCTION. Iowa Agr. Expt. Sta. Bull. 202, pp. [11]-24, illus. 1922.

⁴ DUNN, L. C. A GENE FOR THE EXTENSION OF BLACK PIGMENT IN DOMESTIC FOWLS. Amer. Nat. 56: 404-406. 1922.

Hays³ in his proposed theory on the inheritance of color in the Rhode Island Red, suggested that three genes are responsible for the plumage pattern. The autosomal gene B for reddish-brown pigment, in combination with either a sex-linked gene L for color pattern and gold color or an autosomal gene E for the extension of melanic pigment, is responsible for the color phenotypes found in this breed. Hays did not attempt to account for the minor fluctuations which he suggests are probably due to the action of modifying factors.

The purpose of this study was to analyze, insofar as the available data will permit, the color inheritance in the Single Comb Rhode Island Red. The data upon which the analysis was made were taken from the Kansas Agricultural Experiment Station flock of this breed. The matings involved were made for the study of other characteristics in the fowl, but along with other records detailed color descriptions were also recorded for each individual. It was originally planned that this study would be preliminary to later ones where matings would be made for the specific purpose of the analysis of color inheritance. It now seems necessary to terminate the work on this project with the present paper.

MATERIALS AND METHODS

The stock used in this study had been handled and bred for some time as a production flock of Single Comb Rhode Island Reds, and an effort had been made to maintain fairly good standard qualities. Although there was considerable variation among individuals in this flock, the average color had been kept a good dark red.

In order to maintain uniformity and accuracy in classification of eye and plumage color, all descriptions were based on a set of color standards for each region considered.

The standards for feather color were the result of selection, after an examination of a large number of birds, of five gradations in color as being representative of the range of feather colors found in this breed. These feather standards served for classifying both surface and under color. The standard was made by mounting on a white cardboard samples of plucked feathers. Grade 1 was the lightest shade of color while the successive grades indicated increasing intensity of color. The standard colors of plumage and the standard for eye color were those used in a previous experiment by the senior writer.⁶

The descriptions of primary and secondary wing feathers follow a grading system based on the amount of black pigment found on the surface of the feathers of this region. The second full-length primary and the second secondary flight feathers were used for recording the amount of black. The numbers 1 to 6 are used to designate increasing amounts of black. Grade 6 occurred so infrequently that it was included in grade 5 in the summaries, thus only five grades were required in the tables. The range of variations found is shown in figure 1.

The eye color grades were based on the amount of red pigment in the iris. The iris color of each bird was compared with a color chart

³ HAYS, F. A. INHERITANCE OF PLUMAGE COLOR IN THE RHODE ISLAND RED BREED OF DOMESTIC FOWL. *Genetics* 11: [355] 371. 1926.

⁶ WARREN, D. C. THE INHERITANCE OF RHODE ISLAND RED CHICK DOWN-COLOR VARIATIONS AND THEIR RELATION TO COLOR VARIATIONS IN ADULT PLUMAGE. *Jour. Agr. Research* 39: 781-794, illus. 1926.

painted from representative specimens of grades of pigmentation. The grades ranged from an iris with practically no red to one showing a deep reddish bay color.

All descriptive observations were made at maturity (6 months of age), and the birds were graded according to the color standards for the respective regions. In order to secure uniformity in grading, all descriptions were made under natural lighting conditions and by the same person. In the description of the plumage color, the back, hackle, and breast were graded separately. In the study of under color, only the shade of red was considered.

Rather than preserve whole skins from so large a number of birds, feather samples from different regions of the plumage were preserved on cards as previously described by Warren.⁷ Complete records were kept on all birds available for the breeding seasons 1927 to 1930, inclusive, totaling over 3,000 individuals.

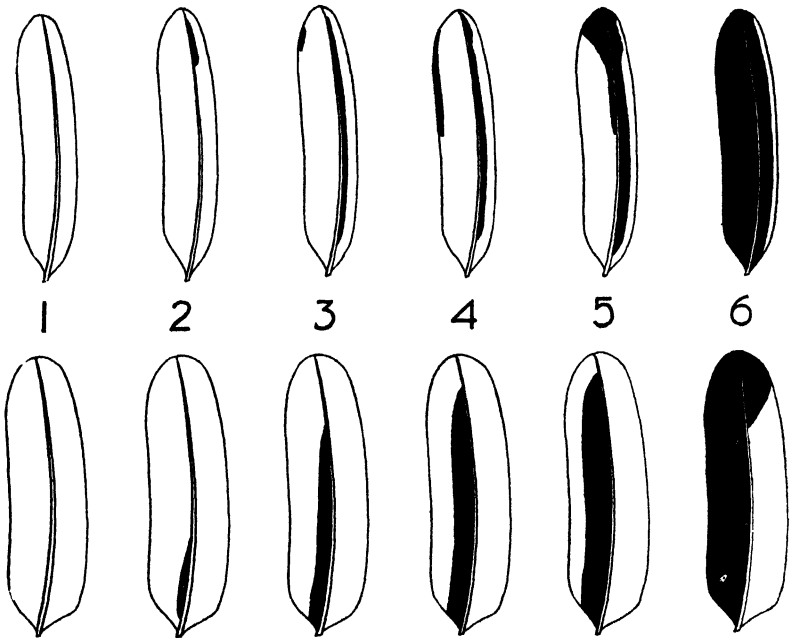


FIGURE 1 - (Grade-number standards for variations in the amount of black pigment in the primary (upper series) and secondary (lower series) wing feathers of adult Rhode Island Red chickens

RESULTS

In the study of color inheritance in various regions of the plumage the results were summarized on the basis of parental color combinations. The color grades of the offspring from the various paternal and maternal grades are shown in table 1. This made it possible to compare the results of mating the lightest colored female with the lightest colored male and darkest color with darkest color as well as reciprocal matings between color extremes. Because of the fact that birds in the extreme grades occurred infrequently and were mated

⁷ WARREN, D. C. See footnote 6.

still less frequently, it was not possible to present the results of all color combinations. The various combinations of color grades were presented in the following tables insofar as they were available:

TABLE 1.—Mean results from all available combinations of parental plumage and eye color grades

BACK PLUMAGE

Sires' color grade (plumage)	Mean grade for daughters when the dams' color grade was—					Mean	Mean grade for sons when the dams' color grade was					Mean
	1	2	3	4	5		1	2	3	4	5	
5		2 08	3 19	3 35		3 22		4 44	4 33	4 43		4 38
4	2 75	2 71	2 99	3 10	3 61	3 07	3 87	3 88	4 17	4 05	4 11	4 07
3			2 89	2 97		2 94			4 05	4 15		4 12

BREAST PLUMAGE

5		2 44	2 44	2 75		2 66		3 38	3 91	3 58		3 65
4	2 08	2 27	2 52	2 95	3 41	2 61	2 77	3 23	3 42	3 55	3 46	3 37
3		2 22	2 43	2 41		2 33		2 97	3 17	3 34		3 15
2	1 18	2 17	2 07	2 45		2 14	2 50	2 55	2 90	2 91		2 79

HACKLE PLUMAGE

5		3 22	3 60	3 68		3 59		3 88	3 96	4 17		4 00
4		2 89	3 31	3 32	3 93	3 32		3 29	3 77	3 82	4 22	3 79
3		3 19	3 18	3 37	3 46	3 27		3 38	3 60	3 66	3 47	3 59
2		2 88	3 13	3 26		3 14		3 35	3 26	3 56		3 41

UNDER PLUMAGE

5		2 42	2 33	2 64		2 45		3 09	2 99	2 60		2 91
4	2 39	2 48	2 46	2 81	3 09	2 09	2 46	2 71	2 76	2 77	2 98	2 76
3		2 49	2 69	2 72		2 61		2 85	3 10	2 96		2 96
2	2 59	2 30	2 54	2 61		2 50	2 75	2 72	2 64	2 81		2 74

BLACK IN PRIMARIES

5	2 73	2 68	2 93	3 21		2 84	3 33	3 42	4 06	4 26		3 68
4	2 39	2 49	2 83	2 90		2 63	3 33	3 42	4 02	3 72		3 61
3	2 05	2 54	2 66	2 88		2 60	3 00	3 20	3 39	3 40		3 35
2	1 42	1 93	2 14	2 83		2 04	2 40	2 54	2 92	3 78		2 75

BLACK IN SECONDARIES

5	3 14	3 11	3 33	3 52	3 90	3 35	3 95	4 06	4 09	4 32	4 19	4 13
4	2 30	2 82	2 92	3 18	3 31	2 87	3 23	3 50	3 87	4 33	4 17	3 81
3	2 39	2 18	2 16	2 46		2 27	2 62	3 49	3 35	3 50		3 22
2	1 91	2 22	2 52	2 91	2 63	2 39	2 53	2 86	3 38	3 67	3 91	3 20
1	1 66	1 57	2 31			1 87	1 92	2 21	2 73			2 22

EYE COLOR

6			9 39	9 23		9 27		9 43	8 74			8 75
5	9 40	9 20	9 88	9 56	9 63	9 55	10 00	9 52	9 85	9 82	10 00	9 80
4	9 69	9 22	9 38	9 52	9 36	9 39	9 33	9 55	9 44	9 64	9 69	9 47
3	8 75	9 27	9 09	9 46	9 30	9 24	8 64	9 41	9 07	9 24	9 27	9 17

* These means are for classes of less than 30 individuals.

The inheritance of the shade of red color was studied in four regions of the plumage: Back (mid-dorsal region); breast; hackle, which is the collar of long pointed feathers covering the base of the neck; and the under surface, which includes the fluff at the base of the feather. The under color was that of the mid-dorsal region. There is frequently considerable contrast in the shade of red in these different regions, and for that reason they were considered separately. The breast is frequently lighter in color while the hackle color may be either lighter or darker than the general surface color. The under color is usually considerably lighter than that of the surface.

The inheritance of the amount of black in the wing feathers was studied, and the results from the primary and secondary wing feathers were tabulated separately. In addition to the studies on plumage color the inheritance of eye color was also considered.

INHERITANCE OF BACK COLOR

Since the back color of the males averaged somewhat darker than that of the females, it was necessary to tabulate separately the results from the two sexes. Any study of back color is complicated by this sexual dimorphic condition, because a male and female of the same genetic make-up are not the same color.

The results from the matings of individuals varying in back color are shown in tables 1 and 2. In table 1 are given the mean grades of sons and daughters from varying parental grades for all surface regions. These condensed results show the general trends but are not satisfactory for genetic analyses. In table 2 is presented the distribution in the various matings of the offspring among the five grades of back color.

TABLE 2.—*Distribution of offspring and mean results from all available parental combinations of grades of plumage and eye color*

BACK PLUMAGE

Parental color grade	Distribution of daughters in color grades indicated						Distribution of sons in color grades indicated					
	1	2	3	4	5	Mean grade	1	2	3	4	5	Mean grade
♂ × ♀												
5×4		16	71	72		3.35			4	57	53	4.43
5×3		21	142	63		3.19			6	96	60	4.33
5×2	1	9	35	10		2.98			3	13	18	4.44
Total or average	1	46	248	145		3.22			13	169	131	4.38
4×5		2	60	94	2	3.61		1	7	101	24	4.11
4×4		65	292	113	1	3.10		1	48	272	70	4.05
4×3		6	77	228	85	2.99		2	28	203	87	4.17
4×2		6	62	92	19	2.71	1		35	100	20	3.88
4×1			6	13	1	2.75			2	13		3.87
Total or average	14	212	685	312	4	3.07	1	4	120	689	201	4.07
3×4			15	40	13	2.97			4	39	12	4.15
3×3			8	23	4	2.89			2	14	3	4.05
Total or average			23	63	17	2.94			6	53	15	4.12

TABLE 2.—*Distribution of offspring and mean results from all available parental combinations of grades of plumage and eye color—Continued*

BREAST PLUMAGE

Parental color grade	Distribution of daughters in color grades indicated						Distribution of sons in color grades indicated					
	1	2	3	4	5	Mean grade	1	2	3	4	5	Mean grade
5×4	7	30	25	21	1	2.75		5	21	31	5	3.58
5×3	5	9	9	4		2.44		1	6	10	6	3.91
5×2		6	2	1		2.44		1	4	2	1	3.38
Total or average	12	45	36	26	1	2.60		7	31	43	12	3.65
4×5		6	25	32		3.41	1	2	15	17	2	3.46
4×4	17	62	102	78	2	2.95		24	70	99	21	3.55
4×3	27	107	96	32	1	2.52	1	25	94	78	20	3.42
4×2	50	130	89	19		2.27		45	117	86	9	3.23
4×1	8	18	11			2.08		11	15	4		2.77
Total or average	102	323	323	161	3	2.61	2	107	311	284	52	3.37
3×4	14	84	60	12		2.41		21	59	52	8	3.34
3×3	5	83	54	8		2.43	1	19	63	25	8	3.17
3×2	34	125	74	6		2.22	1	48	79	31	7	2.97
Total or average	53	292	188	26		2.33	2	88	201	108	23	3.15
2×1	5	32	22	6		2.45	1	16	27	9	2	2.91
2×3	11	45	16			2.07		13	18	9		2.90
2×2	8	20	13	1		2.17		21	13	4		2.55
2×1	14	3				1.18		5	2	1		2.50
Total or average	38	100	51	7		2.14	1	55	60	23	2	2.79

HACKLE PLUMAGE

5×4			11	23		3.68		1	3		9	4.17
5×3		1	33	50	1	3.60		1	13	46	12	3.96
5×2			7	2		3.22			2	5	1	3.88
Total or average		1	51	75	1	3.50		2	18	62	22	4.00
4×5			5	19	3	3.93				7	2	4.22
4×4	4	28	139	120	5	3.32		11	47	123	31	3.82
4×3		14	133	86		3.31		3	55	84	23	3.77
4×2		6	19	3		2.89		1	8	5		3.29
Total or average	4	48	296	228	8	3.32		15	110	219	56	3.79
3×5			21	18		3.46		1	15	16		3.47
3×4		12	154	118		3.37		15	84	120	29	3.66
3×3		27	203	83		3.18		13	117	140	26	3.60
3×2		13	57	30	1	3.19		8	29	33	1	3.38
Total or average		52	435	249	1	3.27		37	245	309	56	3.59
2×4		4	98	41		3.26		1	53	64	2	3.56
2×3		13	69	27		3.13		18	43	29	7	3.26
2×2	2	11	35	8		2.88		9	22	15	6	3.35
Total or average	2	28	202	76		3.14		28	118	108	15	3.41

TABLE 2.—Distribution of offspring and mean results from all available parental combinations of grades of plumage and eye color—Continued

Parental color grade	Distribution of daughters in color grades indicated						Distribution of sons in color grades indicated					
	1	2	3	4	5	Mean grade	1	2	3	4	5	Mean grade
♂ × ♀												
5×4.....		6	7	1		2.64		2	3			2.60
5×3.....		14	7			2.33		4	12	2		2.89
5×2.....		7	5			2.42			10	1		3.09
Total or average.....		27	19	1		2.45		6	25	3		2.91
4×5.....		15	74	25		3.09		12	71	10		2.98
4×4.....	4	50	107	23		2.81	4	39	88	14		2.77
4×3.....	7	69	65	4		2.46	1	31	48	11		2.76
4×2.....	1	50	46	1		2.48		34	64	4		2.71
4×1.....	1	15	12			2.39	4	22	24	2		2.46
Total or average.....	13	190	304	53		2.69	9	138	295	41		2.76
3×4.....	2	40	73	9		2.72		12	97	7		2.96
3×3.....	11	83	185	15		2.69		22	137	43		3.10
3×2.....	11	155	160	8		2.49	1	61	170	24		2.85
Total or average.....	24	278	418	32		2.61	1	95	404	74		2.96
2×4.....		70	82	8		2.61		26	97	2		2.81
2×3.....		35	35	2		2.54		29	37	4		2.64
2×2.....	7	73	45			2.30		30	73	1		2.72
2×1.....		7	10			2.50		2	6			2.75
Total or average.....	7	185	172	10		2.50		87	213	7		2.74

BLACK IN PRIMARIES

5×4.....		13	39	17	6	3.21		2	11	6	27	4.26
5×3.....	1	43	82	15	10	2.93		3	24	22	35	4.06
5×2.....	8	104	105	27	6	2.68	2	47	58	69	36	3.42
5×1.....		6	7	2		2.73		1	5	2	1	3.33
Total or average.....	9	166	233	61	22	2.84	2	53	98	99	99	3.68
4×4.....	2	15	22	8	3	2.90		8	13	10	16	3.72
4×3.....	3	25	32	5	7	2.83		4	11	25	20	4.02
4×2.....	8	58	41	6	4	2.49	1	22	43	41	18	3.42
4×1.....	5	22	10	1	3	2.39		9	7	4	7	3.33
Total or average.....	18	120	105	20	17	2.63	1	43	74	80	61	3.61
3×4.....	3	20	28	14	2	2.88	2	3	6	18	18	4.00
3×3.....	4	44	38	12	3	2.66	1	23	21	25	17	3.39
3×2.....	13	101	97	15	3	2.54	5	57	52	54	26	3.20
3×1.....	5	9	4	1		2.05	2	7	3	3	4	3.00
Total or average.....	25	174	167	42	8	2.60	10	90	82	100	65	3.35
2×4.....		7	20	2		2.83		7	5	8	12	3.78
2×3.....	15	57	21	4		2.14	3	29	31	16	6	2.92
2×2.....	58	206	35		1	1.93	10	105	49	28	10	2.54
2×1.....	3	7	1			1.82		7	2	1		2.40
Total or average.....	76	277	77	6	1	2.04	22	148	87	51	28	2.75

TABLE 2.—Distribution of offspring and mean results from all available parental combinations of grades of plumage and eye color—Continued

BLACK IN SECONDARIES

Parental color grade	Distribution of daughters in color grades indicated						Distribution of sons in color grades indicated					
	1	2	3	4	5	Mean grade	1	2	3	4	5	Mean grade
3×5		1	7	16	6	3.90	1	1	4	7	14	4.19
5×4	2	4	48	35	13	3.52	1	2	8	26	40	4.32
5×3	4	36	102	89	19	3.33	1	8	27	55	60	4.09
5×2	13	10	39	23	12	3.11	3	2	14	31	32	4.06
5×1	32	7	20	8	2	3.14	2		3	9	8	3.95
Total or average	19	58	216	171	52	3.35	8	13	56	128	154	4.13
4×5	4	1	16	3	8	3.31	2		3	3	12	4.15
4×4	4	14	75	36	5	3.18		3	12	45	56	4.33
4×3	29	64	143	69	14	2.92	10	23	58	89	99	3.87
4×2	16	29	51	28	5	2.82	11	14	27	34	30	3.50
4×1	32	37	36	10	4	2.30	13	14	17	28	16	3.23
Total or average	85	145	321	146	36	2.87	36	54	117	199	213	3.81
3×4	6	4	12	1	1	2.49	2		1	8	1	3.50
3×3	15	18	13	5		2.16	2	4	23	18	4	3.45
3×2	13	9	12	4		2.18	6	3	6	17	9	3.49
3×1	12	8	19	5		2.39	8	5	15	4	2	2.62
Total or average	46	39	56	15	1	2.27	18	12	45	47	16	3.22
2×5	1	3	3		1	2.63	1		3	2	5	3.91
2×4	2	6	8	4	2	2.91	2	1	8	5	8	3.67
2×3	18	20	32	7	5	2.52	7	8	17	13	16	3.38
2×2	12	16	19	2		2.22	6	6	7	6	4	2.86
2×1	14	12	5	3		1.91	9	9	3	5	4	2.53
Total or average	47	57	67	16	8	2.30	25	24	38	31	37	3.20
1×3	5	10	9	2		2.31	2	5	4	3	1	2.73
1×2	8	4	2			1.57	6	2	3	3		2.21
1×1	19	10	5	1		1.65	13	4	4	2	1	1.92
Total or average	32	24	15	3		1.87	21	13	11	8	2	2.22

EYE COLOR

6×5	1	16	22	19	7	9.23	3	13	16	3	3	8.74
6×4		3	6	8	1	9.34		3	1	2		8.83
Total or average	1	19	28	27	8	9.27	3	16	17	5	3	8.75
5×6	1	4	30	24	14	9.63		3	12	18	18	10.00
5×5	5	23	64	75	22	9.56		12	48	56	43	9.82
5×4	1	9	51	96	17	9.88	1	13	41	78	45	9.85
5×3	1	18	25	37	4	9.29		10	24	33	8	9.52
5×2		3	13	13	1	9.43	1	1		3	5	10.00
Total or average	8	54	183	245	98	9.55	2	34	123	188	119	9.80
4×6	1	15	41	22	14	9.36	1	7	20	28	15	9.69
4×5	3	24	69	79	34	9.52	1	14	53	69	26	9.64
4×4	4	37	71	82	24	9.38	4	28	54	72	22	9.44
4×3	1	15	34	29	7	9.22	1	8	23	26	13	9.55
4×2	2	5	6	2	1	8.69		2	6	2	2	9.33
Total or average	11	101	224	202	80	9.39	7	53	162	197	78	9.47
3×6		6	5	6	3	9.30		4	5	4	2	9.27
3×5	2	14	31	39	12	9.46	1	29	25	30	6	9.24
3×4		37	54	30	10	9.09	4	26	30	18	11	9.07
3×3		11	19	14	5	8.27	2	5	12	7	8	9.41
3×2	1	4	4	3		8.55		6	4	1		8.55
Total or average	3	72	113	92	30	9.24	7	61	73	63	27	9.17

The usual method of ascertaining in the fowl whether sex-linked factors are involved in a genetic make-up is to compare the female offspring from reciprocal matings. The sexual dimorphism encountered here makes this somewhat difficult. The means would indicate that males and females of the same genotype differ about one grade, the males being the higher. Selecting an average male (grade 4) and an average female (grade 3) and comparing the means when they were mated to the full range of grades of the opposite sex would supply some evidence as to whether sex-linked factors are involved. Since the female bird has only one sex chromosome and thus must receive it from her father she will show the influence of the sex-linked factors which he carries. Thus where the males are of a variable constitution, the daughters should show a greater range of variability than those from the cross where the females are of varying constitution. The mean grades of daughters from the mating of grade 3 females to the range of grades of males are no more variable than are those of daughters from the matings of grade 4 males to females of the different grades. Further evidence may be obtained from the comparison of the reciprocal mating of the most extreme—the darkest males with the lightest females and the lightest males with the darkest females. These combinations were $5\sigma \times 2\varphi$ and $3\sigma \times 4\varphi$, and the former had daughters averaging 2.98, and the latter 2.97 in color grade. Thus there appears to be no evidence that sex-linked factors are involved in producing the color variations on the back of the Rhode Island Red.

The rather consistent gradation of the means (for back color) shown in table 1 indicates somewhat strongly that variations in the surface color are inherited. The males used in the matings were of the grades 3, 4, and 5, while the females were distributed in all five classes. As the grade of the male decreased when mated to one grade of females, the mean of the offspring decreased. The same results were obtained from the different grades of females.

Although the evidence for heritability of the shade of red on the back appears to be good, it seems that the mode is not simple. The wide range of color grades among the off-spring in all crosses recorded in table 2 signifies that a number of determiners are probably responsible for the standard color.

INHERITANCE OF BREAST COLOR

The plumage of the breast region is usually lighter in color than that of the rest of the body (table 1). A comparison of the means of offspring from all available matings for back color and breast color shows that the males manifest a slightly greater difference in the two regions than the females.

Since the males were somewhat darker than the females with respect to breast color, the sexes were recorded separately. In table 2 the reciprocal crosses, grade $4\sigma \times$ grade 2φ and grade $2\sigma \times$ grade 4φ , show the female offspring from the lighter males to be slightly darker than those from the darker males. Since this is the reverse of what would be expected from sex-linked factors, it may be stated that the sex chromosomes probably carry no genes affecting color in this region. There is also a slight difference in the mean of the males from these two combinations of matings, and this is not to be expected in cases of sex linkage. The difference in the means of offspring color from

these reciprocal matings is probably due to individual differences in the parent stock.

From the data in table 2 it is evident that the range of offspring from all available matings is relatively wide. In matings of grade 5♂ with grade 4 ♀ all five grades of female and four grades of male offspring were produced. This indicates that several factors are involved, and that many of the parents were not homozygous for the dark breast which they carried. Since the means of offspring from the different matings (table 2) seem to be lower than, rather than intermediate to, the parent grades, the major factors for light pigmentation are probably partially dominant to those for dark pigmentation.

TABLE 3.-- *Coefficient of correlation of color intensity in various sections of plumage*

Sections of plumage	Number of birds	Region A		Region B		Correlation coefficient
		Mean	Standard deviation	Mean	Standard deviation	
Back (A) and breast (B).....	1, 022	3. 11	0. 691	2. 41	0. 835	0. 787±0. 008
Back (A) and hackle (B).....	1, 022	3. 11	. 691	2. 24	. 610	. 660±. 012
Back (A) and secondaries (B).....	1, 022	3. 11	. 691	2. 84	1. 041	-. 013±. 021
Primaries (A) and secondaries (B).....	1, 022	2. 53	. 903	2. 84	1. 041	. 679+ . 012

The data in table 3 show a positive correlation of 0.787 ± 0.008 between the grades of breast and back color. The probable explanation of this correlation is that the same major genetic factors control color in these two regions, although minor factors might cause some independent variation. The fact that the breast is usually lighter than other regions may be due to a general pattern factor.

INHERITANCE OF HACKLE COLOR

The difference found between the intensity of color in the hackle of the two sexes was less than that observed in the sections of the plumage previously discussed. From the values in table 2 it may be noted that when fowls in grades 4 and 5 were mated together they produced very few offspring lighter than grade 3, whereas when both parents were in grade 2 they produced several dark offspring. These results indicate that light color is dominant over dark since the heterozygotes would give the dominant color a wider range. The more restricted distribution may be the result of fewer factors being involved than in the case of the other regions studied. The inheritance of hackle color is probably dependent on more than one pair of factors, since no simple ratios can be assigned to the results.

An examination of the means from reciprocal matings, grade 5♂ × grade 3 ♀ and 3♂ × 5 ♀, or 4♂ × 2 ♀ and 2♂ × 4 ♀, show no indication of sex linkage. In one case the darker male gives the darker female offspring, and in the other case the darker male produces the lighter female offspring. These results indicate that genes in the sex chromosomes are not responsible for the variations in color of the hackle.

A positive correlation of 0.660 ± 0.012 (table 3) was found between the hackle and back color. The relatively high association of color intensity in these two regions would indicate that any existing differences are due to pattern factors. Another explanation of the correlation is that the same factors are responsible for the color of both regions and there are few modifying factors present to cause variations.

INHERITANCE OF UNDER COLOR

The study of inheritance of under color is based primarily on the red pigmentation in the fluff of the feathers on the back and does not take into consideration the black or white in this region. The variety of colors found in this part of the feather makes it very difficult to grade this region and may account for some of the irregular results obtained. The males seem to be slightly darker than the females (table 2), but there appears to be little order to the mean grade of offspring from different grades of males.

There is so little difference in average color grade from the different parental combinations that the evidence for inheritance of variations in under color is not good. These results may be due to unidentified environmental factors and also may be due to the narrow range of variation found. The narrow range in average color grade from the different parental combinations is evident from the results in table 2, there being little difference in the mean of offspring from grade 5 males and that of grade 2 males. There was a general tendency for the under color to vary with the surface color.

INHERITANCE OF BLACK IN PRIMARY AND SECONDARY WING FEATHERS

The major genetic factor considered in the inheritance of black in the primary and secondary wing feathers is the e^m factor of Dunn.⁸ This factor acts by restricting the expression of melanic pigment to the extremities and is commonly known as the Columbian pattern. Dunn believes that its normal allelomorph is extended black. The variations studied here are probably due to modifying factors influencing the degree of restriction of black in the flight feathers. The amount of black in the web of the feathers of these two regions varies widely; some feathers are practically without black, while others are almost entirely black as seen in figure 1 which shows the range of the variations. The grade 6 occurred so infrequently that the few were included in grade 5 in the tables.

Upon examination of the means of offspring recorded in table 2 it is seen that there is more black in the secondaries than in the primaries. This difference may be due to a pattern factor or to a different combination of the factors affecting each region. Sexual dimorphism necessitated the recording of the sexes separately.

Most of the matings summarized in table 2 show a wide range of grades of offspring. This fact would indicate the possibility that several factors are responsible for the inheritance of black pigment in these two regions. The means of the offspring from the various matings, as recorded in table 2, are generally intermediate between the two parental grades, thus indicating a blending type of inheritance without much indication of dominance of either the lack or excess of melanic pigment. The possibility of the same factors controlling color in primaries and secondaries is supported by the correlation coefficient of 0.679 ± 0.012 between the degree of black in the two sections of the wing. The correlation-coefficient value is given in table 3.

The data in table 2 show the amount of black in the secondaries of the female offspring from the combination, grade 2♂ × grade 5♀ to be less than that from the combination, grade 5♂ × grade 2♀. This might be taken as evidence for sex linkage in the secondaries,

⁸ DUNN, L. C. See footnote 4.

but it is rather doubtful that this could be the case after an examination of crosses $1\sigma \times 3\varphi$, $3\sigma \times 1\varphi$, $2\sigma \times 4\varphi$, and $4\sigma \times 2\varphi$ in which the female offspring do not seem to follow the mean of the male parent. The means of the male offspring in these reciprocal crosses differ to such an extent as to make more questionable the possibility of sex linkage. The means of female offspring from reciprocal matings $4\sigma \times 2\varphi$, $2\sigma \times 4\varphi$, and $1\sigma \times 3\varphi$, $3\sigma \times 1\varphi$ (table 2) show no indications of sex linkage in the primary wing feathers.

The means of the offspring from all mating combinations recorded in table 2 indicate that excess black in these two areas is inherited, because the means seem to recede as the mean of the mating combination is reduced. The means of the offspring have a direct relation to the grade of the male parent if a comparison is made of varying grades of males when mated to females of the same grade. The same is true if the female grade is varied while her mates' grades are held constant. The rather wide difference between the mean of offspring from the darkest and lightest males indicates the possibility of fewer factors being concerned or, at least, a more clean cut segregation of those involved.

The distribution of color grades for the offspring (table 2) includes all five grades in most cases and bears out the earlier statement concerning a wide range in the amount of black in the primaries and secondaries of different birds.

Bittenbender⁹ stated that individuals carrying a dark-red color often have an excess of black in wing. This would indicate that black in wing is associated with the intensity of surface pigmentation. Table 3 data show a negative correlation of -0.013 ± 0.021 to exist between the intensity of surface pigmentation and the degree of black in secondaries. A correlation of such small magnitude is not significant and shows that there is no association between the degree of pigmentation of these two regions.

The amount of black in the wing may be controlled by factors which modify the Columbian pattern found in the Rhode Island Red, and these factors probably have nothing in common with those controlling the intensity of red in the surface.

INHERITANCE OF EYE COLOR

Upon examining the eyes of Rhode Island Red fowls, one will find all variations in color, from a light gray (grade 1) to the reddish-bay (grade 6) iris which is most desired. Differences in distribution as well as kinds of pigment in the eye made the classification of the variations difficult. The grades indicated primarily the amount of red in the iris. The eye color standards¹⁰ show that the reduction in red color begins at the margin of the pupil and progresses toward the outer margin of the iris. Grade 1 occurred so infrequently that no provision was made for this group in the tables. Observations also indicated that there was no change in eye color associated with age. The eye color variations were not associated with any variations in plumage color.

Bond¹¹ in describing eye color in several breeds states that daw eye (no red) acts as a recessive to a black pigment iris in pigeons and

⁹ BITTENBENDER, H. A. See footnote 3.

¹⁰ WARREN, D. C. See footnote 6.

¹¹ BOND, C. J. ON CERTAIN FACTORS CONCERNED IN THE PRODUCTION OF EYE COLOUR IN BIRDS. *Jour. Genetics* 9: [69]-81. 1919.

fowls. Further work on the genetics of eye color in poultry is not available.

An examination of table 2 discloses no pronounced sexual dimorphism with regard to eye color. The results here are much less definite than those obtained from the study of black in flight feathers. It is also seen that there is a slight tendency for the mean grade of the offspring to vary with that of the parents. There is, however, considerable lack of consistency in the agreement. Although there is a rather wide range of eye color in the stock considered, the means of eye color for offspring from the most extreme parental combinations do not differ greatly.

The general trend of the results may be taken as evidence of some heritability of eye-color variation in the Rhode Island Red. The evidence is less definite than that found for any of the other color factors studied except that for under color. This may be taken to indicate that some environmental factors are acting to obscure the heritable tendencies. No such environmental factors were identified.

DISCUSSION OF RESULTS

It has not been possible to place the variations in plumage and eye color in the Rhode Island Red on a definite genetic factorial basis. Such results are more or less to be expected when dealing with a quantitative character of this type. The results are further complicated by the necessity of classifying the variations in arbitrary grades.

The method by which the standard color has been acquired in any breed of fowl would tend to bring about a complicated genetic constitution. The poultryman has set up an ideal and accumulated within the breed any hereditary variations which have produced a change in the direction of the ideal. This method would tend to establish in the breed a number of modifying factors. The accumulation of these factors has proceeded over a considerable period of years, and anyone who attempts to unravel the skein of germ plasm should expect to find it a much tangled one.

All the birds considered in this study had a color characteristic of the breed, and the variations considered were those which measured the closeness of approach to the ideal color. It is well known that two major factors are concerned in the production of the Rhode Island Red pattern. These are the sex-linked gold which determines the ground color, and the factor for Columbian pattern which acts in restricting the expression of black to the extremities. It is evident that we have well fixed in this breed other factors darkening the gold color, since the buff varieties, which are much lighter in color, are also known to carry the gold factor. The factors which have been considered are those affecting the intensity of the gold color, and they are not well fixed in the breed. The factors influencing the amount of black in the flight feathers are those modifying the restricting action of the factor for Columbian pattern on black.

The fact that the intensity of color in the various sections of the plumage has been considered separately does not necessarily indicate that color in these regions shows independent inheritance. The back, hackle, breast, and under surface were the sections of the plumage which showed greatest contrast in color. The general re-

sults tend to support the hypothesis that one major factor controls color in all the regions mentioned. The correlation studies showed a fairly high degree of association in the intensity of pigmentation for these sections of the plumage. The summaries of the results in all cases show the males to be darker than the females. Also wherever there was any evidence for dominance or recessiveness the lighter color appeared to be dominant. It is suggested that the difference in intensity of color in the various sections of the plumage may be due to the action of a general pattern factor in the breed.

Although the exact number of factors concerned in color inheritance in this breed could not be determined, there is definite evidence that intensity of color in the back, hackle, and breast, and the amount of black in the flight feathers are inherited. The evidence favoring the inheritance of intensity of color in the under surface of the plumage is much less definite.

In the case of the amount of black in the flight feathers there is a very close agreement in the mean grade of the parents and that of the offspring. The results seem to supply unquestionable evidence that variations in the amount of black in these feathers are inherited but also indicate that several factors are probably concerned in their transmission. Correlation studies show that there is no association between the intensity of pigmentation in the general body color and the amount of black in the wing feathers.

The variations in eye color in this breed do not show definite evidence that they are inherited. The general trend of the results seems to suggest their heritability, but the difference in results when extremes of color are mated is not great.

In all sections of plumage studied and also in eye color, the matings were of such a nature that tests could be made for evidence of sex linkage of the factors concerned. In no case is there evidence that any of the factors involved had their genes carried by the sex chromosome.

SUMMARY

Although there are differences in the shade of red color in four sections of the plumage the general surface, hackle, breast, and under surface of the Single Comb Rhode Island Red fowl, no evidence was found for important independent factors affecting color in these different regions.

Evidence was found for the heritability of the variations in intensity of pigmentation of the general surface, hackle, and breast, but the results support the view that it is a multiple-factor situation. The evidence for heritability of under color was not so definite.

Variations in amount of black in the flight feathers were found to be inherited, but the degree of black in the wings was found to be independent of the intensity of pigmentation of the general surface.

Results on the study of eye color did not seem to indicate that the variations in eye color are inherited.

Sexual dimorphism was observed in plumage-color variations, the males averaging darker than the females in all sections.

None of the characteristics studied showed any evidence that sex-linked factors are concerned.

RECOVERY OF PHOSPHORUS FROM PRAIRIE GRASSES GROWING ON CENTRAL OKLAHOMA SOILS TREATED WITH SUPERPHOSPHATE¹

By H. F. MURPHY²

Associate agronomist, Department of Agronomy, Oklahoma Agricultural Experiment Station

INTRODUCTION

The fact that soils differ considerably in their ability to fix phosphorus from an applied phosphate fertilizer has been known to soil investigators for some time. The efficiency of such fertilizers has been recognized as being low, although actual recovery figures as determined from plant yields and analyses are not very numerous in the literature. Buie³ estimated that only 10 to 35 percent of the additional phosphoric acid added to the cotton crop was utilized the first year. Russell (7, 8)⁴ presents data which indicate a very low recovery from several phosphorus carriers on some English grasslands. Penetration tests by different workers (2, 3, 4, 6, 9, 10) indicate that most of the phosphorus from an applied phosphate fertilizer never moves a great distance away from the point at which it is applied, although in light soils and in soils receiving manure penetration is greater.

It is the purpose in this paper to present some data on the removal of phosphorus by native prairie grass from virgin soils treated with superphosphate and other fertilizers. Data are also presented on the penetration of phosphorus in one of these soils as measured by the amount present in a more or less easily soluble form in different parts of the profile.

THE SOIL AREAS

Two different soils, one common in the central cross timbers of Oklahoma and the other common in the central prairie section, in which there is slightly less rainfall, were selected for these studies. The soils had apparently never been disturbed from their virgin state, and on each was growing a uniform stand of native prairie grass. *Andropogon scoparius* Michx. was the dominant species, but *A. furcatus* Muhl., *Panicum virgatum* L., *Sorghastrum nutans* (L.) Nash, and *Bouteloua hirsuta* Lag. were common in the area.

The central cross-timber soil selected is a rather dark friable loam at the surface, but changes to a yellowish-brown color at a depth of 3 to 5 inches. The subsoil is a friable clay and is underlain at a depth of 2 to 3 feet with yellowish-brown sandstone. The dark color of the surface is due to the accumulation of organic material from the native vegetation. This soil has not been given a series name as yet, but

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² The writer acknowledges his indebtedness to B. F. Kiltz and H. A. Daniel for assisting in some of the collecting and in the analytical work, and to Dr. H. J. Harper for criticisms and suggestions in the preparation of the paper.

³ BUIE, T. S. Private communication.

⁴ Reference is made by number (italic) to Literature Cited, p. 916.

is rather common in the east-central portion of the State. The surface soil contains 0.055 percent total nitrogen, 0.019 percent total phosphorus, and 0.0115 percent replaceable potassium. The replaceable potassium was determined by the neutral normal ammonium acetate method. This soil is acid in reaction and is not very productive.

The central prairie soil is classified as Vernon sandy loam, shallow phase. It has a friable clay subsoil and is underlain with sandstone at a depth of about 3 feet. The surface 3 inches is neutral to slightly acid, the 3- to 12-inch horizon is slightly acid, while at about 12 inches and below the soil is slightly basic in reaction. It is considered only fairly productive under the usual climatic conditions common to the area. Both soils are deficient in phosphorus.

METHODS

Two series of plots were laid out on the central cross-timber soil, one early in the spring of 1929, and the other in the spring of 1930. Each plot was one fortieth acre in size (1 rod by 4 rods) and all fertilizer treatments were duplicated, unless otherwise stated. The plots lay side-to-side in each series with one complete set of treatments followed by the duplicate set. The fertilizers were applied broadcast early in April at the beginning of each test and no further applications were made during the tests, which were concluded in the autumn of 1932. The plots on the Vernon soil series were laid out early in the spring of 1930 and were similar in size, treatment, and duplication to those started on the cross-timber area of the same year, except that some duplicated plots of other fertilizer treatments were added and the calcium nitrate treatments were omitted. The plots lay in three tiers with no similar treatments having end-to-end plots. Every sixth plot was used as a check.

The grass was mowed during the latter part of July each year and the field dry weights of hay were recorded. Composite samples of hay from each plot were taken and placed in an electric oven at 105° C. for 48 hours, at which time the samples were weighed and the yields were recalculated to a 10-percent moisture basis. The dried samples were ground to pass through the 0.5-mm sieve of a Wiley laboratory mill and were analyzed for phosphorus. The phosphorus was determined by the official method, magnesium nitrate being used in the preparation of the solution as recommended by the Association of Official Agricultural Chemists (1).

The samples of soil used were composite samples made by mixing equal amounts of soil taken from five different locations on each plot. The samples were collected from the various depths by means of soil tubes. The easily soluble phosphorus content was determined by the fifth-normal sulphuric acid method described by Harper (5, p. 4).

The hydrogen-ion concentration of the soil was determined electrometrically, using the quinhydrone electrode. The dilution was 1 part of soil to 2 parts of water.

RESULTS AND DISCUSSION

A summary of the results, given in tables 1 and 2, indicate as a whole a very small removal of phosphorus by prairie grass from both types of soil. If it is assumed that all the extra phosphorus removed came from the applied superphosphate, the maximum recovery in 4 years was 24.7 percent. The smallest recovery in 3 years was less than 3 percent. These figures represent the total gain in removal of phosphorus over check or nitrogen plots, but apparently this extra phosphorus came almost if not entirely from the applied superphosphate. There was very little difference between the percentage of phosphorus in the hay grown on the plots treated with nitrogen alone and in that grown on the check plots. Furthermore, the percentage of phosphorus in the hay grown on the plots that received phosphate alone and in that grown on the plots that received phosphate in combination with nitrogen was practically the same when the same amount of phosphorus was applied. In general, the use of superphosphate in addition to nitrogen increased the yield of hay. Such was not the case, however, with cyanamid on both soils nor with urea on the Vernon soil, where the yields were not so large as when nitrogen was used alone; yet the percentage of phosphorus was of the magnitude expected for phosphorus treatments.

TABLE 1. —Removal of phosphorus from central cross-timber soil by prairie grass where different rates and ratios of nitrogen and phosphorus were used

Treatment per acre (pounds)	Total hay yield for 4 years	Phos- phorus in hay	Phos- phorus removed	Phos- phorus added	Phos- phorus re- covered ^a in 4 years
	Pounds	Percent	Pounds	Pounds	Percent
Checks (average of 5 plots)	8,824	0.0567	5.002	0	0
100 ammonium sulphate+50 superphosphate ^b	8,795	.0603	5.306	4.366	6.96
100 ammonium sulphate+100 superphosphate ^b	9,157	.0607	5.557	8.732	6.36
100 ammonium sulphate+200 superphosphate ^b	9,461	.0736	6.963	17.465	11.23
300 15 15-15-15 ^c	10,951	.0885	9.688	19.648	23.85
300 nitrate of soda	11,305	.0702	7.942	17.465	16.83
200 superphosphate					
100 muriate of potash					
500 2.12:6 ^c	9,665	.0795	7.686	26.197	10.24
300 superphosphate	10,413	.0854	8.890	26.197	14.84
300 ammonium sulphate+150 superphosphate	11,739	.0756	8.882	13.099	24.70
300 ammonium sulphate+300 superphosphate	13,913	.0820	11.417	26.197	22.03
300 ammonium sulphate+600 superphosphate	14,010	.0929	13.025	52.394	14.08
300 ammonium sulphate	10,042	.0562	5.646	0	0

^a The term "recovered" is used here and in table 2 to indicate the difference between the amount of phosphorus removed from the nitrogen and phosphorus plot and from the nitrogen plot, where data are available. Where there is no similar nitrogen treatment the check-plot data are used. This difference is compared with the quantity of phosphorus added to the soil in the fertilizer which gives the percentage indicated in column 6. It is recognized that such a comparison may be subject to errors because some plants have more extensive root systems than others and hence greater soil volume in which to secure nutrients as well as perhaps an increased ability to secure plant foods from crude material, yet, by making use of the nitrogen plots in determining the differences in uptake of phosphorus, these are probably taken care of as well as experiments of this type can afford. It is certain that the recovery from the applied superphosphate does not exceed the percentage given.

^b The superphosphate contained 20 percent P_2O_5 .

^c The first number refers to the percentage of nitrogen, the second to percentage of P_2O_5 , and the third to the percentage of K_2O in the fertilizer.

TABLE 2.—The 3-year removal of phosphorus from central cross-timber soil by prairie grass and from Vernon sandy loam

CROSS-TIMBER SOIL

Treatment ^a	Total yield of hay per acre	Phos- phorus in hay	Phos- phorus removed	Phos- phorus added	Phos- phorus recovered in 3 years
	Pounds	Percent	Pounds	Pounds	Percent
Check (average of 4 plots)	5,531	0.0428	2,365	0	0
Calnitro	7,334	.0440	3,293	0	0
Ammonium sulphate	7,117	.0442	3,144	0	0
Nitrate of soda	7,068	.0438	3,097	0	0
Calurea	7,040	.0399	2,809	0	0
Urea	6,667	.0419	2,794	0	0
Cyanamid	7,777	.0453	3,525	0	0
Calcium nitrate	7,597	.0404	3,070	0	0
Calnitro+superphosphate	8,216	.0545	4,481	17,465	6.80
Ammonium sulphate+superphosphate	8,982	.0550	4,943	17,465	10.30
Nitrate of soda+superphosphate	8,859	.0540	4,788	17,465	9.68
Calurea+superphosphate	7,867	.0545	4,289	17,465	8.47
Urea+superphosphate	7,286	.0565	4,114	17,465	7.56
Cyanamid+superphosphate	7,061	.0568	4,014	17,465	2.88
Calcium nitrate+superphosphate	8,774	.0504	4,420	17,465	7.73

VERNON SANDY LOAM

Check (average of 8 plots)	4,635	0.0537	2,500	0	0
Calnitro	4,063	.0401	1,875	0	0
Ammonium sulphate	4,624	.0488	2,257	0	0
Nitrate of soda	5,418	.0485	2,630	0	0
Calurea	5,326	.0450	2,397	0	0
Urea	6,268	.0530	3,324	0	0
Cyanamid	5,798	.0491	2,835	0	0
Calnitro+superphosphate	5,817	.0664	3,845	17,465	11.39
Ammonium sulphate+superphosphate	5,923	.0617	3,658	17,465	8.02
Nitrate of soda+superphosphate	5,761	.0555	3,197	17,465	3.25
Calurea+superphosphate	6,038	.0664	4,012	17,465	9.25
Urea+superphosphate	6,089	.0677	4,125	17,465	4.69
Cyanamid+superphosphate	5,499	.0674	3,709	17,465	5.00
Superphosphate	5,052	.0635	3,211	17,465	3.56
15:30:15, 267 pounds per acre	6,658	.0833	5,550	34,929	9.09
0:46:0, 364 pounds per acre	5,805	.1019	5,920	83,027	4.01
11:46:0, 364 pounds per acre	7,406	.0958	7,099	83,027	5.69
10:30:10, 400 pounds per acre	7,404	.0822	6,086	52,394	7.08
12:24:12, 333 pounds per acre	6,815	.0819	5,596	34,929	9.19

^a The average phosphorus content of the hay for the treatments shown are: Checks 0.0428 percent; all nitrogen treatments 0.0429 percent, and all nitrogen and phosphorus combinations 0.0545 percent.

^b All nitrogen fertilizers were applied at the rate of 40 pounds of nitrogen per acre. The superphosphate contained 20 percent P_2O_5 and was applied at the rate of 200 pounds per acre.

^c The average phosphorus content of the hay for the treatments shown are: Checks, 0.0537 percent; all nitrogen treatments, 0.0484 percent; superphosphate alone, 0.0635 percent; and all nitrogen and superphosphate combinations, 0.0642 percent.

When nitrogen was applied with superphosphate there was an increase in yield as compared with that when superphosphate alone was used; nevertheless, the percentage of phosphorus in the hay remained practically the same. In the surface 12 inches of untreated cross-timber virgin soil (table 3), the easily soluble phosphorus, i.e., the phosphorus soluble in 0.2 N H_2SO_4 , was less than that in the treated soil when both nitrogen and phosphorus were applied, but below this portion of the profile there was little difference. The surface few inches of Vernon soil 2 years after the application of superphosphate showed (table 4) more soluble phosphorus than did the untreated soil. For most of the treatments the increase was confined mostly to the surface 3 inches. It thus appears that as nitrogen is furnished to the plant to increase yield, the plant makes use of the small extra amount of more easily soluble phosphorus left in the surface few inches of soil by the applied superphosphate, and so the percentage of phosphorus recovered from the fertilizer is increased.

The increase in yield is probably the result of many factors. It is suggested that a larger root system may contribute considerably in this respect. If such is the case it is probable, though not proved, that the root system becomes more extensive in the surface soil volume instead of extending down into the subsoil of low fertility. It does not appear that the nitrogen influenced phosphorus absorption except as it might have increased the yield of hay, since the percentage of phosphorus in the hay for any of the nitrogen treatments was of the same order of magnitude as that in the hay produced on unfertilized soil.

No significance is to be attached to the difference in the amount of phosphorus removed in the case of the various nitrogen-phosphorus combinations. The yields of the plots and duplicates were as uniform as could be expected, but with so small a removal as was secured in any one case slight variations in yield would account for the differences obtained. This is emphasized by the fact that no one nitrogen carrier proved best on the two types of soil studied.

TABLE 3 — *The pH and soluble phosphorus at various depths 2 years and 3 years after application on the central cross-timber soil*

2 YEARS

Treatment per acre	0-3 inches		3-6 inches		6-12 inches		12-24 inches		24-36 inches	
	pH	Solu- ble phos- phorus	pH	Solu- ble phos- phorus	pH	Solu- ble phos- phorus	pH	Solu- ble phos- phorus	pH	Solu- ble phos- phorus
		P p m		P p m		P p m		P p m		P p m
Check.....	6.44	3.0	6.48	2.5	6.44	1.0	6.39	1.4	6.58	1.2
Calnitro+superphosphate.....	6.63	11.0	6.63	1.0	6.59	1.0	6.34	1.0	6.35	1.6
Ammonium sulphate+superphosphate.....	6.42	5.0	6.35	1.0	6.32	2.0	6.20	2.0	6.86	1.4
Nitrate of soda+superphosphate.....	6.58	4.0	6.58	1.5	6.34	3.0	5.83	1.2	6.42	1.0
Calurea+superphosphate.....	6.27	3.5	5.59	1.5	5.61	3.0	5.78	1.5	5.78	1.2
Urea+superphosphate.....	6.44	6.0	6.04	1.0	5.96	3.0	5.88	1.0	5.86	1.5
Cyanamid+superphosphate.....	6.35	3.5	5.99	1.5	5.99	.5	6.09	Trace	6.04	1.5
Calcium nitrate+superphosphate.....	6.04	3.0	6.13	1.5	5.80	3.0	5.82	1.7	5.96	2.0
Check.....	6.30	3.0	6.40	1.5	6.00	Trace	5.80	1.2	6.01	1.2

3 YEARS

Check.....	6.23	3.0	6.04	2.5	5.54	1.0	5.24	0.8	5.47	1.5
100 pounds ammonium sulphate, 50 pounds superphosphate.....	6.34	3.0	6.28	3.0	5.76	2.0	5.33	1.5	5.57	2.0
100 pounds ammonium sulphate, 100 pounds superphosphate.....	6.18	3.0	6.30	1.0	5.75	1.5	5.78	1.5	5.68	1.0
100 pounds ammonium sulphate, 200 pounds superphosphate.....	6.35	4.0	5.71	2.0	5.75	1.0	5.61	1.2	5.86	1.0
300 pounds 15:15:15 fertilizer.....	6.30	3.0	6.56	2.5	5.86	1.5	5.35	1.5	5.92	1.0
Check.....	6.08	3.0	6.70	2.0	6.01	1.0	6.49	1.2	5.57	1.0
300 pounds nitrate of soda, 200 pounds superphosphate, 100 pounds muriate of potash.....	6.30	5.0	6.09	2.0	5.76	1.5	5.71	.8	5.70	1.2
300 pounds ammonium sulphate, 150 pounds superphosphate.....	6.11	4.0	5.71	2.5	5.54	2.0	5.83	1.5	5.71	1.0
400 pounds ammonium sulphate, 300 pounds superphosphate.....	6.30	3.5	6.11	1.0	5.75	1.5	5.33	.8	5.40	1.0
300 pounds ammonium sulphate, 600 pounds superphosphate.....	5.96	9.0	5.94	1.5	5.37	1.5	5.12	1.0	5.23	1.7
Check.....	6.09	3.5	5.57	2.0	5.61	1.5	5.54	1.0	(^b)	(^b)
300 pounds superphosphate.....	6.11	3.0	5.88	2.0	5.96	(^c)	6.06	1.2	(^b)	(^b)
500 pounds 2:12:6 fertilizer.....	6.32	4.0	6.04	1.7	5.86	1.5	6.37	1.5	(^b)	(^b)

^a The composite sample of the soil at the beginning of the experiment had a pH of 6.78 and contained 3 parts per million of soluble phosphorus in the surface 3 inches.

^b No samples were taken on account of sandstone.

^c Trace.

TABLE 4.—The soluble phosphorus content of the surface 6 inches of Vernon soil 2 years after applying the fertilizers

Treatment	Phosphorus soluble in 0.2 N H ₂ SO ₄	
	0-3 inches	3-6 inches
Checks (average of 8 plots).....	<i>P. p. m.</i> 8	<i>P. p. m.</i> 6
Calnitro+superphosphate.....	8	8.5
Ammonium sulphate+superphosphate.....	13	5
Nitrate of soda+superphosphate.....	12	10.5
Calurea+superphosphate.....	11.5	6.5
Urea+superphosphate.....	11	11
Cyanamid+superphosphate.....	10.5	6.5
Superphosphate.....	11.5	6.5

SUMMARY AND CONCLUSIONS

This paper reports results secured in field trials to determine the quantities of phosphorus removed from two common pasture and grassland soils in central Oklahoma by native prairie grasses consisting mainly of *Andropogon scoparius*. The natural phosphorus content of these grasses is low.

The use of superphosphate on these soils increased the yield of hay and raised the phosphorus content of the hay produced. When nitrogen as well as phosphorus was applied in the fertilizer the percentage of phosphorus in the hay was of the same order of magnitude as when superphosphate only was applied.

When nitrogen and superphosphate were applied the yield of hay in all cases was greater than when superphosphate alone was used, but it was not always greater than when nitrogen was used alone. The percentage of phosphorus in the hay produced on the plots that received nitrogen alone was of the same order of magnitude as that in the hay produced on unfertilized soil.

Regardless of whether the soil furnished any extra phosphorus for the superphosphate, nitrogen treated plots beyond that furnished on the nitrogen plots, the recovery of phosphorus from the applied superphosphate was very small.

If the amount of phosphorus soluble in 0.2 N H₂SO₄ can be taken as a guide, the phosphorus applied did not penetrate to any great distance in the cross-timber soil, being confined largely to the surface 3 inches; certainly it did not penetrate to a depth exceeding 12 inches.

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THE NUTRITIVE VALUE OF ALFALFA LEAVES AND STEMS¹

By JERRY SOTOLA²

Assistant professor and assistant animal husbandman, Washington Agricultural Experiment Station

INTRODUCTION

Many stockmen are convinced that the leafier as well as the greener hays possess higher nutritive value than those which are stemmy or discolored. Workers in nutrition have observed that stemmy hay, when fed alone, frequently results in excessive body losses in both calcium and phosphorus, whereas the feeding of leafier grades of hay generally results in favorable balances.

Different cuttings of alfalfa are affected by soil conditions, soil moisture, available irrigation water, temperature, relative humidity, and such other factors as age of stand, varietal differences, and differences in plant maturity. The texture also influences the curing qualities. Three cuttings made at the Washington Irrigation Branch Station at Prosser showed that the third cutting had the most leaves, and that although the stems of the first cutting were coarsest, the percentage by weight of stems was highest in the second cutting. First-cutting hay as produced under irrigation in the Yakima Valley during cool, moist weather may contain some weeds, such as cheat. The second cutting is produced during a short, hot growing period with low relative humidity. The third cutting is sometimes affected by the earliness or lateness of cold weather in the fall (33).

For those reasons, a separation of stems and leaves was undertaken to determine how each part of the plant functions in animal nutrition. The blossoms were included with the leaves.

PURPOSE OF THE INVESTIGATION

The experiments reported in this paper were planned to measure any existing differences in the three cuttings of alfalfa hay or their stems and leaves insofar as feeding value is concerned. Such a study should show the desirability of proper handling during the curing process, directed toward a maximum retention of those qualities important in feeding. It should further be of value in establishing grades of alfalfa hay based on true values if considered from the feeding standpoint.

The coefficients of apparent digestibility of the organic nutrients of the stems, leaves, and whole hay of the three cuttings, and the biologi-

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cal values of the proteins in the hay, in the leaves, and in the stems were determined. The gain or loss of calcium or phosphorus to lambs fed stems, leaves, or whole hay as sole rations should supply information as to their value as sources of these important minerals.

REVIEW OF LITERATURE

Headden (17)³ showed that 50 percent of the weight of the alfalfa plant is leaves, and that they contain 80 percent of the crude protein and only 22 percent of the crude fiber.

Haag (8) showed that the limiting factor for growth of rats fed alfalfa-leaf proteins was cystine. Hart and Humphrey (11, 12) compared alfalfa proteins in efficiency with those of the corn grain for milk production and found these to be practically equal. Nevens (27) fed alfalfa proteins to rats at a 9 percent level of intake and found the biological value of these proteins to be 62. Mitchell and Hamilton (24), reviewing the work of Hamilton, Nevens, and Grindley, show that of the total nitrogen in alfalfa hay, 8 percent is arginine, 3.9 percent histidine, 4.4 percent lysine, 1 percent cystine, and 19.1 percent is nonprotein nitrogen.

Hart et al. (14) observed that a more liberal storage of calcium was obtained when liberally milking cows were on fresh green alfalfa than when they were on dry alfalfa. Steenbock et al. (39) showed that hay cured in the field and kept under caps produced calcium equilibrium when fed to cows in full milk flow, in quantities of 10 pounds, in addition to corn silage and grain. Hay cured in the windrow for 4 days with exposure to air and light resulted in negative calcium balances when fed in the same manner. Smith and Briggs (35) have shown that alfalfa cured in the dark is deficient in antirachitic potency and that synthesis of vitamin D occurs when the alfalfa is exposed to sunlight in the curing process. Alfalfa carefully spread out on the field from 11:15 a.m. one day to 12 m. the next, during which time the sun shone 15 hours and 5 minutes, possessed mild calcifying powers. The hay which lay in the swath for 1 week and was exposed to sunshine for 57.3 hours and to 0.37 inches of rain was found to be highly antirachitic.

Russell (32) showed that the Mason process employing artificial heat produced dried alfalfa leaves with a vitamin A potency seven times greater than that of the same product cured in the field. However, the leaves when cured artificially contained only small quantities of the antirachitic vitamin. When the alfalfa was dried in the sun, without exposure to dew or rain, there was an increase in the antirachitic potency of the leaves, but a decrease in vitamin A content.

Bethke and Kick (4) report that exposure of alfalfa hay to the sun, rain, or dew over a period of several days resulted in a marked loss of vitamin A. Hauge and Aitkenhead (16) consider vitamin A to be probably the most important single nutritive factor in alfalfa that may be affected by curing.

Hathaway, Davis, and Graves (15) showed that under the conditions of their experiment artificially cured alfalfa was twice as potent in vitamin A as was the field-cured hay.

Recent work by Smith and Briggs (34) on the vitamin A content of alfalfa leaves shows that a loss of 20 to 33 percent in this factor occurs by allowing the alfalfa to be spread out carefully for 2½ hours,

³ Reference is made by number (italic) to Literature Cited, p. 942.

from 11:15 a.m. to 2 p.m. No greater degree of destruction occurred when the time of exposure was increased to 6½ hours in the same day. No loss of green color in the leaves could be observed in comparison with leaves cured in the dark, with which the other samples were compared in respect to vitamin A potency.

The same workers show that a 75-percent loss of vitamin A results when the alfalfa is allowed to lie in the field under the same conditions overnight and until 8 a.m. the next day. This loss was increased to 84 percent upon further exposure until 12 m. the second day. Alfalfa severely bleached as a result of 1 week's exposure to sun and rain in the field while in the swath retained but 4 percent of the vitamin A present, as compared with the sample cured in the dark.

Douglas, Tobiska, and Vail (5) working under Colorado conditions found that hay cut at the early bloom stage contained more vitamin A than did that cut at other stages of growth, the method of curing being the same. The hay cured in diffused light was superior in vitamin A content in most cases to that cured in direct sunlight.

Douglas, Tobiska, and Vail (5) also found that second-cutting hay was lower in vitamins B and G than that of the first or third cuttings. When the third crop reached the early bloom stage before frost, it usually rated second in nutritive value. The inferiority of the second crop was attributed to the heat of the summer, with excessive transpiration and woodiness of plant tissue.

In the above studies rats were used as the experimental animals. In another phase of the work, in which guinea pigs were used, it was shown that green alfalfa contains an abundance of vitamin C, practically all of which was lost when the plants were cured for hay.

Hunt et al. (19) point out that hays vary in their "quality-producing" factors, depending upon the time of harvesting and method of curing. Vitamin B is present in such small amounts in hays as to be of small importance. A high protein and a high vitamin G content are correlated in a general way with a good green color and leafiness. As the greenness fades, due to the stage of maturity of the plant, the protein and vitamin G content also decrease while the fiber increases.

Meigs, Hartman, and Converse⁴ recently have pointed out a tendency toward parallelism between vitamin A content and color in both alfalfa hay and butter. The color of butter seemed to be more influenced by feed than by the breed of the cow.

Meigs and Converse (22) stress good quality hay for best milk production and reproduction of dairy cows.

That quality of hay may be influenced by the curing processes employed is clearly shown by Willard (42) and Westover (41) who stated that alfalfa leaves do not assist in withdrawing moisture from the stems in the curing process, thus supporting the view of Higgins (18). Alfalfa apparently cures at least as rapidly with the leaves removed as with them attached.

Kiesselbach and Anderson (20, 21) state that the supposed transpiratory function of the leaves in alfalfa severed from its roots is a negligible factor in the curing process. Prolonged curing in the swath resulted in loss of leaves through shattering and also produced bleaching, while windrowing and cocking relatively green plants

⁴ MEIGS, E. B., HARTMAN, A. M., and CONVERSE, H. T. VITAMIN A AND COLOR IN HAY AND MILK. U.S. Dept. Agr., Bur. Dairy Indus., 4 pp. 1933. [Mimeographed.]

materially extended the curing period. Judicious partial swath curing to hasten the rate of drying without bleaching followed by windrowing and by prompt storage when cured, seemed the best practices.

Higgins (18) showed that during clear hot weather, alfalfa dried in the swath to a moisture content of approximately 25 percent was badly bleached and that the leaves were very brittle, a large proportion of them being lost. Hay dried in the swath lost 25.3 percent in weight when subjected to shaking as compared to losses of 20.7, 21.6, 22.3, and 16.6 percent for hay dried in the swath in the shade, in the windrow and not turned, in the windrow and turned, and that dried in the cock, respectively. The ordinary mechanical loss of leaves in field curing alfalfa hay is placed by Headden (17) at 15 to 20 percent.

Rather (30) stressed the importance of getting the hay out of the swath and into the windrow within a few hours after it was cut. Willard (42) and Westover (41) supported this view, and showed that bleaching and loss of leaves by shattering and crumbling were thereby reduced.

Headden (17) stated that overcuring resulted in the loss of leaves and stems, amounting to one fifth to two thirds of the crop.

Kiesselbach and Anderson (20, 21) and Parker (28) stressed purity, texture, and high percentage of leaves, clinging foliage, green color, and pliable stems as essentials of high-quality hay, whereas Rather (30) emphasized green color, pleasant aroma, and retention of leaves.

Haag and his coworkers (10) explain the negative nitrogen balances obtained when alfalfa hay alone was fed to dairy cattle, as due to a faulty energy intake on account of bulk, a low biological value of the proteins, and a low phosphorus content in turn supposedly related to protein metabolism. For dairy cows a sole-alfalfa-hay ration does not seem to supply adequate phosphorus and sufficient total digestible nutrients, except for moderate milk production.

The calcium-phosphorus ratio of alfalfa is reported to vary from 3 to 1, up to 25 to 1. The work of Meigs, cited by Haag (8), favors a 2 to 1 calcium-phosphorus ratio, and later a 1.5 to 1 ratio. Haag and his coworkers (9) in a later paper state that apparently the storage of calcium is conditioned by the relatively low phosphorus intake.

Woll (43) cites instance in which dairy cows, beef cattle, sheep, and even hogs frequently receive nothing but alfalfa throughout the year, either as pasture or soilage in the summer, or as hay in the winter. Apparently no ill effects resulted from this monotonous diet.

METHOD OF EXPERIMENTATION

PLAN OF THE WORK

Three cuttings of northern-grown common alfalfa were studied by feeding the stems, whole hay, and leaves of each cutting to a different group of six lambs. Owing to the length of time, approximately 100 days, that each of the six lambs was confined to experimental feeding of each cutting of hay, it was possible to study only one cutting each year to avoid feeding old hay. The following order was followed with each cutting:

1. Preliminary period
2. Nearly nitrogen-free ration

3. Preliminary period
4. Stems of alfalfa hay
5. Preliminary period
6. Whole alfalfa hay
7. Preliminary period
8. Leaves of alfalfa hay
9. Preliminary period
10. Nearly nitrogen-free ration

Experimental periods of 10 days (36, 38) were preceded by preliminary periods of 10 days. In view of the nearly nitrogen-free feeding, periods 1 and 9 were of 20 days duration to accustom the lambs to the unusual rations. During all experimental tests the lambs were confined in specially constructed metabolism crates (36).

Stems and leaves, including blossoms, were separated by hand from the first, second, and third cuttings. Approximately 500 pounds each of stems, leaves, and blossoms, and of whole alfalfa hay were used. The stems and hay were fed chopped.

METHOD OF SAMPLING AND ANALYSIS

The samples of feed were thoroughly mixed, and samples were then taken for chemical study. The feces and urine were treated by the method of French (7) to prevent loss of nitrogen. Official methods (3) of analysis were used.

HAY SAMPLES USED

In 1929 first-cutting alfalfa at the half-bloom stage (36) was placed in a barn loft while still green within a 2-hour period after cutting and cured slowly in subdued light. In November, the leaves, including the blossoms, were separated from the stems. Representative samples for chemical analysis were taken. In 1930 a sample was taken from the second cutting and separated into stems and leaves. The following year the third cutting was handled similarly. These were cured under the same conditions as the first cutting.

The results of the mechanical separations expressed in percent, on the basis of the weight of air-dried hay, are shown in table 1.

The first and third cuttings, according to the official hay grades (29), would rate as U.S. No. 1 extra leafy, and the second cutting as U.S. No. 1 alfalfa on the basis of leafiness. Although no color charts were used, all of the hay samples, because of the method of curing, would have graded as extra green.

TABLE 1.—Percentage of stems and leaves in the alfalfa hay studied

Cutting	Leaves	Stems
	Percent	Percent
First.....	50.27	49.73
Second.....	45.82	54.18
Third.....	65.61	34.39

LAMBS AND THEIR BODY WEIGHTS

A different group of six cut-back range lambs was used each year. The initial and final weights are reported in table 11. Each year the lambs were selected in pairs, one pair being light, another medium,

and a third fairly heavy in weight. As far as could be determined, the lambs in all groups were sired by Hampshire rams and out of Lincoln-Merino crossbred ewes.

In 1929 there were 5 ewe lambs and 7 wethers in the group; in 1930 only wethers were used; and in 1931, 7 ewes and 5 wethers were used. The individual records make it possible to trace sex differences. Losses in weight by lambs during periods of nearly nitrogen-free feeding were the rule. When stems were fed most lambs lost weight although some gained, and a few showed no change. On whole-hay rations the lambs gained weight except on first-cutting hay. On the leaf rations gains and losses alternated in the first- and second-cutting hay. On the third-cutting rations losses were the rule. Weight fluctuations of lambs during 10-day periods when the animals had access to water at all times are of slight significance, especially when based on single weighings.

RATIONS FED

Along with the nearly nitrogen-free rations, each lamb was fed 30 g of mixed oil daily. The mixture consisted of 1 part cod-liver oil and 3 parts raw linseed oil.

The nearly nitrogen-free mixture contained 460 parts of cut mature Albit wheat straw low in nitrogen, 250 parts of cane sugar, 250 parts of powdered cornstarch, 10 parts calcium carbonate (c.p.) and 10 parts sodium chloride. The dry matter consumed by each lamb during each 10-day period is shown in table 11. The oil was used to raise the caloric value of the ration, so that even during days of low dry-matter intake the energy value of the nearly nitrogen-free ration might be well above the maintenance requirement. This condition must be met so that the fecal and urinary nitrogen excreted during periods of nearly nitrogen-free feeding may be strictly of the minimum endogenous type.

The quantity of roughage consumed is according to Armsby (2) not a factor in digestibility. The dry matter consumed in the form of stems, whole hay, and leaves was fairly constant in 1929, good for stems and leaves but high for whole hay in 1930, almost identical to 1930 figures for whole hay and leaves, but low for stems in 1931. In all cases the lambs were fed these feeds according to their appetites.

ANALYTICAL PROCEDURE

The Gunning modification of the Kjeldahl method was used in determining nitrogen (3). The calcium and phosphorus were determined by official volumetric methods. Special precautions were taken to prepare the samples properly (6).

CHEMICAL COMPOSITION OF ALFALFA HAY SAMPLES

The chemical composition of the stems, whole hay, and leaves of the first, second, and third, cuttings of alfalfa hay are given in table 2. Figures for moisture just as the feed was fed to the lambs in the metabolism crates are given, too. A better comparison can be made if the figures are reduced to a common moisture basis, 10 percent being considered suitable. Such figures are also contained in table 2.

The stems contained more crude protein than is generally supposed, the percentage decreasing from 8.58 in the first to 7.63 in the third cutting. The crude fiber content was high, ranging from 42.83 to 39.74 percent.

TABLE 2. *Chemical composition of stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa*

Part of plant and cutting	Year	Moisture content as fed	Composition on a 10-percent moisture basis							
			Protein (N x 6.25)	Ash	Crude fat	Crude fiber	Nitrogen-free extract	Calcium	Phosphorus	P:Ca ratio
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	
Stems:										
First	1929	11.84	8.58	4.83	1.05	42.20	.33.34	0.755	0.106	1.7.1
Second	1930	12.68	8.29	5.80	1.63	42.83	31.45	.780	.188	1.4.2
Third	1931	13.66	7.63	5.62	1.23	39.74	35.78	1.042	.096	1.10.8
Whole hay										
First	1929	12.19	11.96	6.40	1.46	34.54	35.64	1.447	.135	1.10.7
Second	1930	13.92	14.05	7.96	2.02	36.37	29.60	1.492	.229	1.6.5
Third	1931	18.54	15.81	7.81	2.83	23.53	40.02	2.580	.166	1.15.6
Leaves										
First	1929	13.43	18.31	9.34	2.52	18.01	41.82	2.953	.188	1.15.7
Second	1930	12.54	19.98	9.79	3.12	15.61	41.50	2.286	.281	1.8.1
Third	1931	15.05	19.33	9.16	3.38	15.80	42.33	3.053	.191	1.16.0

In comparing the data for the whole hay with those of ordinary alfalfa hays, it should be kept in mind that the sample studied was not field cured, and that it contained practically all the upper portion of the plant, there being no leaf loss such as occurs in field curing. The percentage of protein increased from the first to the third cutting, the value for the third, 15.81 percent, being especially high. Leafiness of the third-cutting hay was reflected by its low fiber content, 23.53 percent, as compared with 34.54 and 36.37 percent, respectively, for the first and second cuttings.

The leaves contained the highest percentage of protein, which ranged from nearly 20 to 18.31 percent. The crude fiber in the leaves ranged from 15.61 to 18.01 percent. This percentage may of course be affected somewhat by the thoroughness of the mechanical separation.

The calcium and phosphorus in the stems and leaves afforded an interesting comparison. The leaves of the first cutting contained almost four times as much calcium as the stems, but the difference in phosphorus content was not so great.

Ames and Boltz (1) have found 50 percent of the total phosphorus of the alfalfa plant to be in the organic form. While the amount of total phosphorus was found by them to be greater in the first cutting, a greater proportion of it was combined as organic phosphorus in the second cutting.

The relationship of percentages of calcium in stems to that in leaves was only 1 to 3 for the second and third cuttings. The ratio of phosphorus in stems to that in leaves was 1 to 2 in the first and third cuttings and 2 to 3 in the second cutting.

The preceding data become more effective if the percentage distribution of nutrients in the stems and leaves per 100 pounds of hay is computed. Such data for the first, second, and third cuttings are shown in table 3.

It was observed that 67 to 83 percent of the protein was found in the leaves. Therefore, the higher the percentage of leaves in the hay, the higher will be its protein content. This conforms with the results of other workers.

TABLE 3.—*Distribution of nutrients (as percentage of total) per 100 pounds in stems and leaves of the first, second, and third cuttings of alfalfa*

[10-percent moisture basis]

Cutting	Protein (N×6.25)	Ash	Fat	Crude fiber	Nitrogen- free extract	Calcium	Phos- phorus
Stems *							
First	31.67	33.84	29.18	69.86	44.09	20.18	35.89
Second	32.91	41.19	38.18	76.43	47.26	28.75	54.20
Third	17.14	24.33	16.02	56.86	30.69	15.17	20.91
Leaves *							
First	68.33	66.16	70.82	30.14	55.91	79.82	64.11
Second	67.09	58.81	61.82	23.57	52.74	71.25	45.80
Third	82.86	75.67	83.98	43.14	69.31	84.83	79.09

* First cutting of hay contained 49.73 percent of stems by weight and 50.27 percent of leaves, the second cutting 54.18 percent of stems and 45.82 percent of leaves, and the third cutting 34.39 percent of stems and 65.61 percent of leaves.

Ames and Boltz (1) report that 80 percent of the protein in the alfalfa plant is present in the leaves, and that under favorable circumstances the loss of leaves may be 15 percent of the total crop. The nitrogen content of alfalfa was found to be less in the second cutting than in the first, and increased in the third.

The data in table 3 were secured by multiplying the percentages of stems and leaves in the various cuttings by the figures of their respective composition. Obviously from the standpoint of mineral nutrition, the leaves are the most liberal source of calcium and phosphorus.

EFFECT OF ALFALFA LEAVES AND STEMS ON WATER CONSUMPTION AND THE URINARY AND FECAL EXCRETION OF LAMBS

The rations fed, consisting either of alfalfa stems, leaves, or whole hay, afforded an excellent opportunity of studying their effect on water consumption and on fecal and urinary excretions of growing lambs.

The data in table 4 show the diuretic effect as well as the thirst-stimulating qualities of alfalfa leaves.

The lambs consumed 2.12 times as much water as of feed when fed stems of the first cutting, the values for the second and third cuttings being 3.07 and 2.33 times the quantity of feed eaten.

The leaves of first, second, and third cuttings required 4.09, 3.95, and 2.00 times their weight in water. The whole hay ranked intermediate. It is recognized that the external temperature affects water consumption. The feces were most bulky when stems were fed.

The stems, whole hay, and leaves, in order, exerted progressively greater effects on urine excretion. The leaves, on the average, stimulated three and one third times as much urine excretion as the stems.

Leaf rations resulted in urine richer in nitrogen than when stems were fed. The urine of lambs fed leaves of the first cutting contained a total of 2.75 times as much as when stems were fed.

TABLE 4.—*Water consumption and feces and urine excreted per head by 18 lambs when fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa as their sole ration*

Cutting and trial no.	Ration	Total feed eaten	Total dry matter consumed	Total water consumed	Water consumed per gram of food	Total feces	Feces per 100 grams of food consumed	Feces per gram of dry matter
		Grams	Grams	Grams	Grams	Grams	Grams	Grams
First:								
102 and 103	Stems	6,537.5	5,763.3	13,944.2	2.12	7,756.2	118.37	1.34
104 and 105	Whole hay	5,695.0	5,001.0	14,536.3	2.54	3,731.2	65.60	.75
106 and 107	Leaves	6,191.7	5,359.8	25,260.2	4.09	2,609.2	42.21	.49
Second:								
501	Stems	6,229.2	5,721.3	19,423.8	3.11	7,137.2	114.02	1.24
502	Whole hay	8,720.8	7,939.5	27,338.3	3.13	5,993.0	69.00	.76
503	Leaves	6,500.0	6,014.5	25,537.5	3.95	2,535.7	39.40	.43
Third:								
1001	Stems	4,949.0	4,273.5	11,517.2	2.33	7,557.3	152.85	1.76
1002	Whole hay	7,843.3	6,387.0	17,506.8	2.22	5,054.0	64.50	.79
1003	Leaves	7,486.7	6,359.5	15,070.0	2.00	3,251.2	43.43	.52

Cutting and trial no.	Ration	Total volume of urine	Urine per gram of dry matter consumed	Nitrogen in urine	Nitrogen in feces	Nitrogen voided—	
		Cc	Cc	Grams	Grams	In urine	In feces
First						Percent	Percent
102 and 103	Stems	5,096.7	.88	38.28	43.67	46.7±2.64	53.3±2.64
104 and 105	Whole hay	11,914.2	2.30	81.81	36.93	68.9±2.08	31.1±2.08
106 and 107	Leaves	17,444.5	3.26	105.13	44.28	70.4±.87	29.6±.87
Second							
501	Stems	3,215.8	.56	28.70	40.87	41.3±2.90	58.7±2.90
502	Whole hay	11,300.0	1.41	77.52	59.40	56.6±1.27	43.4±1.27
503	Leaves	17,253.3	2.87	156.12	43.20	78.3±.46	21.7±.46
Third							
1001	Stems	3,520.5	.82	28.27	28.27	50.0±3.20	50.0±3.20
1002	Whole hay	8,530.0	1.33	89.08	46.74	65.6±.50	34.4±.50
1003	Leaves	8,585.1	1.35	131.73	48.79	73.0±.47	27.0±.47

Of further interest was the partition of total excretory nitrogen, between the urine and feces. Whether the rations of leaves and stems result in a greater distribution of excretory nitrogen in the feces or urine has partly been answered above. In the case of the stem rations the trend was toward an almost equal partition of the total excretory nitrogen between the urine and feces. For the rations of whole hay, with the exception of the second cutting, approximately 67 percent of the nitrogen excreted was found in the urine. There was no question that the shift of excretory nitrogen was toward the urine when leaves were fed. Since the leaves contributed more digestible nitrogen than did the stems, and since the lamb can use only so much nitrogen at a time, it was logical to find more nitrogen in the urine when leaf rations were fed. This may be directly attributed to the deamination of surplus amino acids.

CHEMICAL COMPOSITION OF THE FECES

A striking variation in the composition of sheep feces as affected by the type of feed is shown in table 5.

The dry matter content of the feces was highest when alfalfa leaves were fed. In fact, the ration exerting the highest diuretic effect resulted in feces of the lowest moisture content.

TABLE 5.—Average chemical composition (percent) of feces of 18 lambs fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa

Part of plant and cutting	Dry matter	Protein (N×6.25)	Crude fiber	Fat	Ash	Nitrogen- free extract
Stems:						
First	42.22	3.59	22.43	0.37	2.85	12.98
Second	41.98	3.66	22.74	.62	3.55	11.41
Third	30.07	2.30	15.28	.55	2.97	8.97
Whole hay:						
First	63.20	6.21	29.18	1.90	6.37	19.54
Second	59.48	6.23	28.62	1.98	6.89	15.76
Third	49.90	5.81	19.18	2.86	9.26	12.79
Leaves:						
First	80.26	10.71	18.34	5.34	17.03	28.84
Second	71.65	10.64	19.30	5.32	12.64	23.75
Third	63.14	9.42	14.44	5.61	13.65	20.02

Feces from lambs fed alfalfa stems contained only 2.85 to 3.55 percent of ash, whereas when leaves were fed the ash varied from 12.65 to 17.03 percent. The leafier grades of hay fed to lambs had a higher fertilizer value.

COEFFICIENTS OF APPARENT DIGESTIBILITY

The usual procedure, as outlined previously (36) was followed in determining the coefficients of apparent digestibility. Table 6 contains such coefficients for the first, second, and third cuttings of hay.

TABLE 6.—Coefficients of apparent digestibility of stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to 6 lambs as their sole ration during 10-day periods

FIRST CUTTING

Part of plant and trial no.	Lamb no.	Dry matter	Crude protein (N×6.25)	Crude fiber	Fat	Ash	Nitrogen- free extract
Stems:							
102.....	100	46	55	39	51	41	55
	101	46	52	40	74	39	55
	102	45	52	39	50	29	54
	103	46	56	36	62	35	59
103.....	104	40	40	32	56	12	56
	105	43	49	35	57	23	56
Average.....		44.3	50.7	36.8	58.3	29.8	55.8
Whole hay:							
104.....	100	53	67	44	19	34	65
	101	59	69	52		40	70
	102	53	64	44	12	31	65
	103	51	64	40	15	32	65
105.....	104	50	63	39	12	34	62
	105	51	64	41	14	30	64
Average.....		52.8	65.2	43.3	14.4	33.5	65.2
Leaves:							
106.....	100	64	78	49	11	34	76
	101	63	76	84		20	62
	102	51	69	38		8	64
	103	65	77	56	17	13	80
107.....	104	62	75	53	72	22	74
	105	63	74	53	19	25	75
Average.....		61.3	74.8	55.5	29.8	21.3	71.8

TABLE 6.—*Coefficients of apparent digestibility of stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to 6 lambs as their sole ration during 10-day periods—Continued*

SECOND CUTTING

Part of plant and trial no.	Lamb no.	Dry matter	Crude protein (N×6.25)	Crude fiber	Fat	Ash	Nitrogen-free extract
Stems:							
501	201	50	56	41	56	41	60
	202	45	54	35	57	45	56
	207	50	57	45	58	27	62
	204	53	57	45	54	41	64
	205	43	41	37	52	13	56
	206	48	40	43	59	21	61
Average		48.2	50.8	41.0	56.0	31.3	59.8
Whole hay:							
502	201	60	72	34	38	43	73
	202	55	70	32	33	48	70
	207	57	72	35	29	45	73
	204	55	71	36	33	35	70
	205	54	68	36	26	34	70
	206	52	66	33	18	41	67
Average		55.5	69.8	34.3	29.5	41.0	70.5
Leaves:							
503	201	68	80	47	55	52	75
	202	66	79	45	40	48	74
	207	72	81	57	35	53	80
	204	71	80	57	29	50	80
	205	69	78	53	28	49	78
	206	72	81	58	28	54	80
Average		69.7	79.8	52.8	35.8	51.0	77.8

THIRD CUTTING

Stems							
1001	1	50	70	42	26	19	61
	2	50	58	41	34	29	61
	3	48	53	40	28	19	60
	4	44	28	38	22		63
	5	46	49	38	30	16	59
	6	46	53	36	36	24	62
Average		47.3	51.8	39.2	29.3	21.4	61.0
Whole hay.							
1002	1	60	73	41	19	17	77
	2	60	73	43	23	16	77
	3	61	74	42	29	17	78
	4	60	74	42	29	11	77
	5	60	74	42	36	13	76
	6	62	76	44	33	18	78
Average		60.5	74.0	42.3	28.2	15.3	77.2
Leaves:							
1003	1	66	76	58	17	32	76
	2	68	78	59	20	36	78
	3	69	78	57	32	35	79
	4	68	78	58	24	23	80
	5	66	76	57	17	28	77
	6	70	79	60	31	35	79
Average		67.8	77.5	58.2	23.5	31.5	78.2

The dry matter of the stems of the first-cutting hay was 16 percent less and that of the leaves 16 percent more digestible than that of the whole hay. The protein was 22 percent less digestible in the stems and 15 percent more digestible in the leaves than in the whole hay.

A similar comparison for fiber and nitrogen-free extract showed the nutrients of the stems to be less, and the nutrients of the leaves to be more digestible than the corresponding nutrients of the whole hay.

Table 6 also shows that in the second cutting, the dry matter of the stems was 13 percent less and the dry matter of the leaves 26 percent more digestible than that of the whole hay. The protein of the stems was 27 percent less digestible and that of the leaves 14 percent more digestible.

The results secured in feeding the third-cutting hay (table 6) again showed the dry matter of the stems to be 22 percent less, and of the leaves 12 percent more digestible than that of the whole hay. A big difference in digestibility between the protein of the stems and of the whole hay was noticed, the stem protein in the third cutting being 30 percent less digestible than that in the whole hay.

The results with each of the 3 cuttings were arranged in table 7 and averaged. The average coefficients of 18 determinations for stems, whole hay, and leaves for all three cuttings were also computed.

TABLE 7.—Coefficients of apparent digestibility of stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to lambs

Part of plant and cutting	Dry matter	Crude protein (N×6.25)	Crude fiber	Fat	Ash	Nitrogen-free extract
Stems:						
First.....	44.3	50.7	36.8	58.3	29.8	55.8
Second.....	48.2	50.8	41.0	56.0	31.3	59.8
Third.....	47.3	51.8	39.2	29.3	21.4	61.0
Average.....	46.6	51.1	39.0	47.9	27.5	58.9
Whole hay						
First.....	52.8	65.2	43.3	14.4	33.5	65.2
Second.....	55.5	69.8	34.3	29.5	41.0	70.5
Third.....	60.5	74.0	42.3	28.2	15.3	77.2
Average.....	56.3	69.7	40.0	24.0	29.9	71.0
Leaves:						
First.....	61.3	74.8	55.5	29.8	21.3	71.8
Second.....	69.7	79.8	52.8	35.8	51.0	77.8
Third.....	67.8	77.5	58.2	23.5	31.5	78.2
Average.....	66.3	77.4	55.5	29.7	34.6	75.9

The stems of the second cutting excelled in digestibility of dry matter, and the stems of the third cutting in protein. The fiber of the second-cutting stems was most digestible, as was the nitrogen-free extract of the stems of the third cutting.

At the Illinois Experiment Station (25) 12 head of lambs weighing from 96.4 to 104 pounds were placed upon a maintenance allowance of alfalfa hay. The coefficients for the nutrients in the alfalfa hay were as follows: Dry substance 55.6, crude protein 67.3, nitrogen-free extract 71, ether extract 19, and crude fiber 26.3. Later work at the same station (26) with 4-year-old ewes fed alfalfa hay and approximately 0.15 pound of linseed meal per head showed that the average digestibility of the nutrients in the hay alone were as follows: Dry substance 64.3, crude protein 73, nitrogen-free extract 78, crude fiber 43, and ether extract 51.7. These results are higher than in the case where alfalfa was fed alone and may be attributed largely to the associative effect of the linseed meal.

These results in the main conform with those of the work reported above.

DIGESTIBLE NUTRIENTS IN ALFALFA STEMS, WHOLE HAY, AND LEAVES OF THE FIRST, SECOND, AND THIRD CUTTINGS

The coefficients of apparent digestibility are of practical significance only when used in determining the digestible nutrients of the stems, leaves, and whole hay.

Such data in table 8 show a wide difference in the stems, whole hay, and leaves. In whole numbers there was 42 percent digestible dry matter and 4 percent of digestible protein in alfalfa stems. Third-cutting hay excelled others in digestible protein. The difference between the protein of the stems of the first and second cutting was small. The stems of second-cutting hay contained the largest percentage of digestible crude fiber and of total digestible nutrients, while the stems of the third cutting contained most nitrogen-free extract. Averages for the stems of all three cuttings are included.

TABLE 8. *Percentage of digestible nutrients in stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to animals*

[10-percent moisture basis]

Part of plant and cutting	Dry matter	Protein (N×6.25)	Crude fiber	Fat	Ash	Nitrogen-free extract	Total digestible nutrients	Nutritive ratio
Stems								
First	39.87	4.35	15.53	0.61	1.44	18.60	39.85	1.8.16
Second	43.38	1.21	17.56	.91	1.82	18.81	42.63	1.9.13
Third	42.60	3.95	15.57	.36	1.20	21.83	42.16	1.9.67
Average	41.95	4.17	16.22	.63	1.49	19.75	41.55	1.8.96
Whole hay								
First	47.52	7.80	14.96	.21	2.14	23.24	46.47	1.4.96
Second	49.95	9.81	12.47	.60	3.26	20.87	44.50	1.3.54
Third	54.45	11.70	9.96	.80	1.20	30.87	54.33	1.3.64
Average	50.64	9.77	12.46	.54	2.20	24.99	48.43	1.3.96
Leaves								
First	55.17	13.70	10.00	.75	1.99	30.03	55.42	1.3.05
Second	62.73	15.94	8.24	1.12	4.99	32.29	58.99	1.2.70
Third	61.05	14.98	9.19	.79	2.89	33.09	59.04	1.2.94
Average	59.65	14.87	9.14	.80	3.29	31.80	57.82	1.2.80

Third-cutting hay excelled the others in the percentage of digestible dry matter, protein, nitrogen-free extract, and total digestible nutrients. This was due largely to its extreme leafiness, which was 65.61 percent. The leafiness was further reflected in a low content of digestible fiber, these two factors being inversely related.

The second-cutting hay was slightly superior to the first cutting in its content of digestible dry matter and protein. The first-cutting hay, however, was higher than the second cutting in digestible crude fiber, nitrogen-free extract, and total digestible nutrients.

Leaves of the second cutting contained the most digestible dry matter and protein. The difference between the total digestible nutrients in the second and first cutting was 6.5 percent in favor of the second cutting.

Averages of all the stems of the three cuttings and of all the leaves, when compared with each other, showed large differences.

A given weight of leaves was almost 3.57 times as efficient as the same weight of stems in supplying digestible protein, and the leaves were less bulky. The leafier the hay, the better source of digestible protein it becomes, irrespective of cutting.

The leaves contained almost a third more per pound of total digestible nutrients than did the stems.

An interesting comparison resulted when the values for the digestible protein in the stems and leaves of the three cuttings were applied to the percentage of stems and leaves in each of the three cuttings.

Such data show that 76 percent of the digestible protein in the first and second cuttings and 88 percent of the third cutting was contained in the leaves. This, of course, was on the basis of the samples as produced at the Washington Irrigation Branch Station.

The work of Hart, Kline, and Humphrey (13) is interesting in this connection. They studied the availability of the dry matter, protein, and calcium in second-cutting alfalfa hay fed to heavy-milking cows. The treatment of the hay was as follows: (1) Dried immediately in a Koon artificial drier; (2) partly dried in the field and then dried in the artificial drier; and (3) completely dried in the field.

The availability of dry matter, protein, and calcium was much alike in the three samples of hay. The high temperature of 480° to 535° C. for 40 seconds to which sample 1 was subjected did not reduce the availability of the nutrients under observation.

NITROGEN METABOLISM STUDIES

The gain or loss of protein can be detected readily by a comparison of the total feed nitrogen and that in the excreta. The values obtained in these trials are given in table 9 and summarized in table 10. A positive balance of nitrogen was found in every period during which alfalfa leaves were fed, irrespective of whether the leaves came from the first-, second-, or third-cutting hay. The largest retention of nitrogen during any 10-day period of leaf feeding was 47.5 g for lamb 3 in trial 1003 fed third-cutting leaves, and the smallest, 12.49 for lamb 3 during trial 106, when leaves of the first cutting were fed. Obviously the best single retention of nitrogen on whole hay occurred when alfalfa of the second cutting was fed, a balance of 68.8 g of nitrogen for lamb 2 in trial 502 being obtained.

TABLE 9.—*Nitrogen balance of 6 lambs when fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa during 10-day periods*

FIRST CUTTING

Part of plant and trial no.	Lamb no.	Feed eaten	Nitrogen consumed	Nitrogen voided in—			Nitrogen balance	Intake stored
				Feces	Urine	Total outgo		
Stems.		<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Percent</i>
102	1	5,925	79.73	36.07	37.55	73.62	+6.11	7.66
	2	6,525	87.83	42.10	51.83	93.93	-6.10	
	3	6,525	87.80	42.29	29.47	71.76	+10.04	18.27
103	4	6,750	90.83	40.25	50.27	90.52	+3.31	3.34
	5	6,750	90.83	54.91	28.99	83.90	+6.93	7.63
	6	6,750	90.83	46.43	31.58	78.01	+12.82	14.11
Average		6,537.5	87.97	43.68	38.28	81.96	+6.01	6.83
Whole hay.								
104	1	6,000	112.03	37.25	145.21	182.46	-70.43	
	2	6,000	112.03	34.43	80.80	115.23	-3.20	
	3	6,000	112.03	40.29	65.96	106.25	+5.78	5.16
105	4	5,390	100.64	35.96	72.65	108.61	-7.97	
	5	5,390	100.64	37.01	59.01	96.02	+4.62	4.59
	6	5,390	100.64	36.62	67.26	103.88	-3.24	
Average		5,695.0	106.34	36.93	81.82	118.75	-12.41	
Leaves.								
106	1	6,000	169.06	37.66	102.59	140.25	+28.81	17.04
	2	6,000	169.06	40.66	108.90	149.56	+19.50	11.53
	3	6,000	169.06	53.66	103.91	156.57	+12.49	7.39
107	4	6,400	180.33	41.61	110.59	152.20	+28.13	15.60
	5	6,450	178.92	45.33	98.43	143.76	+35.16	19.65
	6	6,400	180.33	47.74	106.11	153.85	+26.48	14.08
Average		6,191.7	174.46	44.28	105.09	149.37	+25.00	14.38

SECOND CUTTING

Stems.								
501	1	7,275	98.43	43.33	36.34	79.67	+18.76	19.06
	2	5,100	69.00	31.60	42.54	74.14	-5.14	
	3	8,550	115.68	49.57	44.16	93.73	+21.95	18.97
	4	5,600	75.77	32.88	44.36	77.24	-1.47	
	5	5,425	73.40	43.58	28.15	71.73	+1.67	2.27
	6	5,425	73.40	44.28	37.84	82.12	-8.72	
Average		6,229.2	84.28	40.87	38.90	79.77	+4.51	5.35
Whole hay.								
502	1	9,000	204.66	57.38	89.08	146.46	+58.20	28.44
	2	8,225	187.04	55.58	62.63	118.21	+68.83	36.80
	3	10,425	237.06	65.75	130.57	196.32	+40.74	17.19
	4	8,225	187.04	55.19	81.93	137.12	+49.92	26.69
	5	8,225	187.04	59.74	81.46	141.20	+45.84	24.51
	6	8,225	187.04	62.78	81.88	144.66	+42.38	22.66
Average		8,720.8	198.31	59.40	87.93	147.33	+50.98	25.71
Leaves:								
503	1	6,000	197.53	40.16	124.07	164.23	+33.30	16.86
	2	6,000	197.53	42.31	113.36	155.67	+41.86	21.19
	3	9,000	296.30	57.34	191.67	249.01	+47.29	15.96
	4	6,000	197.53	38.56	133.06	171.62	+25.91	13.12
	5	6,000	197.53	42.87	122.82	165.69	+31.84	16.12
	6	6,000	197.53	37.97	123.31	161.28	+36.25	18.35
Average		6,500.0	213.99	43.20	134.72	177.92	+38.07	16.86

* Average nitrogen balance as a percentage of the average nitrogen consumed.

TABLE 9.—Nitrogen balance of 6 lambs when fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa during 10-day periods—Continued

THIRD CUTTING

Part of plant and trial no.	Lamb no.	Feed eaten	Nitrogen consumed	Nitrogen voided in—			Nitrogen balance	Intake stored
				Feces	Urine	Total outgo		
Stems.		Grams	Grams	Grams	Grams	Grams	Grams	Percent
1001	1	4,194	49.07	14.57	26.40	40.97	+8.10	16.51
	2	5,100	59.72	25.08	29.17	54.25	+5.47	9.16
	3	5,100	59.72	28.34	28.67	57.01	+2.71	4.54
	4	5,100	59.72	43.03	20.64	63.67	-3.95	-----
	5	5,100	59.72	30.32	29.54	59.86	-1.14	-----
	6	5,100	59.72	28.27	35.22	63.49	-3.77	-----
Average		4,949.0	57.94	28.27	28.27	56.54	+1.40	+2.42
Whole hay.								
1002	1	6,900	158.04	43.43	74.31	117.74	+40.30	25.50
	2	7,780	178.19	48.67	91.22	139.89	+38.30	21.49
	3	7,990	183.00	47.98	91.17	139.15	+43.85	23.96
	4	8,010	183.46	47.49	97.04	144.53	+38.93	21.22
	5	8,160	186.90	48.15	88.00	136.15	+50.75	27.15
	6	8,220	188.27	14.72	92.76	137.48	+50.79	26.98
Average		7,843.3	179.64	46.74	89.08	135.82	+43.82	+24.39
Leaves:								
1003	1	5,075	148.19	35.21	92.64	127.85	+20.34	13.73
	2	7,565	220.90	49.44	132.95	182.39	+38.51	17.43
	3	8,070	235.64	50.91	137.21	188.12	+47.52	20.17
	4	8,070	235.64	52.41	149.79	202.20	+33.44	14.19
	5	8,070	235.64	56.24	132.85	189.09	+46.45	19.71
	6	8,070	235.64	48.55	144.93	193.48	+42.16	17.89
Average		7,486.7	218.61	48.70	131.73	180.52	+38.09	+17.42

* Average nitrogen balance as a percentage of the average nitrogen consumed.

TABLE 10.—Nitrogen-balance data, with percentages of intake computed for stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to lambs

Part of plant and cutting	Feed eaten	Nitrogen consumed	Nitrogen voided			Nitrogen balance	Intake stored *
			Feces	Urine	Total outgo		
Stems.	Grams	Grams	Grams	Grams	Grams	Grams	Percent
First	6,538	87.97	43.68	38.28	81.96	+6.01	+6.8
Second	6,229	84.28	40.87	38.90	79.77	+4.51	+5.4
Third	4,949	57.94	28.27	28.27	56.54	+1.40	+2.4
Average	5,905	76.73	37.61	35.15	72.76	+3.97	+5.2
Whole hay:							
First	5,695	166.34	36.93	81.82	118.75	-12.41	-----
Second	8,721	198.31	59.40	87.93	147.33	+50.98	+25.7
Third	7,843	179.64	46.74	89.08	135.82	+43.82	+24.4
Average	7,420	161.43	47.69	86.28	133.97	+27.46	+17.0
Leaves:							
First	6,192	174.46	44.82	105.09	149.37	+25.09	+14.4
Second	6,500	213.99	43.20	134.72	177.92	+36.07	+16.9
Third	7,487	218.61	48.79	131.73	180.52	+38.09	+17.4
Average	6,726	202.35	45.42	123.85	169.27	+33.08	+16.3

* Average nitrogen balance as a percentage of the average nitrogen consumed.

The nitrogen balances were most favorable during the feeding of second-cutting hay, all being positive. There was a marked contrast between the balances secured with the first- and second-cutting crop. The lambs ate less of the first-cutting hay, and the nitrogen intake in consequence was only 54 percent of that of the second-cutting hay rations. Four of the six lambs fed the first-cutting hay lost nitrogen. These were the only cases out of 18 in which whole hay was fed where the balances were negative. Some of the objections to short-time experiments as pointed out by Stewart (40) were overcome by thoroughly accustoming the lambs to the metabolism cages before proceeding with the experiments.

In the case of the stem rations of the first cutting, only 1 negative nitrogen balance was observed, whereas when stems of the second-cutting hay were fed, 3 lambs lost nitrogen. The same was true when stems of the third cutting were fed.

The various rations show negative balances for intakes of nitrogen varying from 60 to 112 g for 10-day periods. On the other hand, a positive balance was obtained with as low a nitrogen intake as 49.07 g for a 10-day period. There is no assurance that a growing lamb fed all the alfalfa hay it will consume will be kept in nitrogen equilibrium.

In the nitrogen-balance data in tables 9 and 10 the last column shows the percentage retention of feed nitrogen. The percentage retention was affected by the excess or deficiency in regard to the requirements for maintenance and growth, by the stage of maturity of the animal, and by the net protein-net energy ratio of the ration.

If the protein requirements for maintenance and growth are met as well as the smaller protein requirement for fattening and wool growth, no further storage of protein takes place, and the excess nitrogen is excreted.

BIOLOGICAL VALUE OF PROTEINS IN LEAVES AND STEMS

Differences in digestibility (38) do not affect the biological values of proteins, and biological values are expected to vary with the use to which the protein is put by the body. The amino-acid make-up of the protein as compared with the several separate requirements for amino acids by the animal account for differences in biological values. For any set of conditions the biological value of a protein is dependent upon its amino-acid make-up. Mitchell (23) pointed out that a lowering in the biological value occurred as the concentration of the protein in the ration increased, irrespective of the quantitative intake. This was true of the stem, whole-hay, and leaf rations studied. Failure to equalize the fiber content of the ration fed is not serious. An underestimation of metabolic nitrogen in a period of protein feeding leads to an overestimation of both the food nitrogen retained and absorbed. Since one is divided by the other in computing the biological value, the error is not considered serious. This fact was brought out in connection with the feeding of alfalfa stems, whole hay, and leaves, which varied in their content of crude fiber.

The data on which the computation of the biological values is based are contained in tables 11 and 12. The order of stems, whole hay, and leaves was followed throughout. The same methods of computation were followed as previously described (26).

TABLE 11.—*Biological values of proteins in stem, leaves, and whole hay of the first, second, and third cuttings of alfalfa when fed to 6 lambs as their sole ration during 10-day periods*

FIRST CUTTING

Ration and trial no	Lamb no. ^a	Body weights			Dry matter feed intake	Nitrogen intake	Fecal nitrogen	Nitrogen in urine	Biological value of protein	Average value
		Initial	Final	Average						
		Kilo-grams	Kilo-grams	Kilo-grams	Grams	Grams	Grams	Grams		
Near-nitrogen free.										
100	100e	24.49	22.68	23.5	1,943.00	2.35	15.24	15.92	-----	-----
	101w	27.22	23.81	25.5	3,290.00	3.98	21.73	8.70	-----	-----
	102e	26.76	24.49	25.6	2,823.00	3.42	18.31	5.55	-----	-----
101	103e	25.40	22.68	24.0	2,618.00	4.38	21.15	11.42	-----	-----
	104e	24.04	21.55	22.8	2,196.00	2.66	18.27	14.22	-----	-----
	105e	21.55	18.14	19.8	2,470.00	2.99	17.82	15.84	-----	-----
Stems										
102	100	19.51	19.73	19.6	5,223.00	79.74	36.07	37.55	-----	-----
	101	22.01	21.77	22.3	5,752.00	87.80	42.10	51.83	48	-----
	102	21.71	21.32	21.5	5,752.00	87.80	42.29	29.47	71	-----
103	103	25.40	24.27	24.8	5,951.00	90.83	40.25	50.27	54	67. ± 4.55
	104	23.59	23.59	23.6	5,951.00	90.83	54.91	28.99	81	-----
	105	21.09	21.55	21.3	5,951.00	90.83	46.42	31.58	80	-----
Whole hay										
104	100	21.55	21.55	21.6	5,269.00	112.03	37.25	145.21	-----	-----
	101	23.13	21.32	22.2	5,269.00	112.03	34.43	80.80	35	-----
	102	22.91	22.23	22.6	5,269.00	112.03	40.29	65.96	42	-----
105	103	27.22	22.68	25.0	4,733.00	100.64	35.96	72.65	33	11 ± 2.27
	104	25.17	22.23	23.7	4,733.00	100.64	37.01	59.01	52	-----
	105	22.23	19.50	20.9	4,733.00	100.64	36.62	67.26	43	-----
Leaves										
106	100	22.24	21.98	22.1	5,194.00	169.06	37.66	102.59	42	-----
	101	22.00	21.75	21.9	5,194.00	169.06	40.60	108.90	38	-----
	102	24.04	23.03	23.5	5,194.00	169.06	52.66	103.91	34	-----
107	103	26.31	25.86	26.1	5,540.00	180.33	41.61	110.59	41	41. ± 1.41
	104	24.95	26.31	25.6	5,497.00	178.92	45.33	98.43	49	-----
	105	23.81	23.13	23.5	5,540.00	180.33	47.74	106.11	41	-----
Near-nitrogen free										
108	100	23.81	21.55	22.7	3,221.00	3.90	14.93	8.89	-----	-----
	101	24.04	22.23	23.1	2,855.00	3.45	13.80	14.95	-----	-----
	102	24.49	21.09	22.8	2,467.00	2.99	10.71	11.05	-----	-----
109	103	28.12	24.49	26.3	3,610.00	4.37	21.50	9.58	-----	-----
	104	26.76	23.36	25.1	2,894.00	3.50	16.70	11.20	-----	-----
	105	24.49	22.00	23.2	2,412.00	2.92	22.18	8.01	-----	-----

SECOND CUTTING

Nitrogen free										
500	201w	22.70	19.50	21.1	1,369.00	1.66	12.94	9.83	-----	-----
	202w	20.40	17.50	19.0	1,451.27	1.76	12.62	7.26	-----	-----
	207w	-----	-----	-----	-----	-----	-----	-----	-----	-----
	204w	21.80	20.00	20.9	2,505.06	3.03	17.21	6.41	-----	-----
	205w	23.60	21.50	22.6	2,419.09	2.93	15.70	8.50	-----	-----
Stems	206w	20.20	18.80	19.5	2,481.95	3.00	17.12	6.20	-----	-----
501	201	24.70	26.80	25.7	6,681.91	98.43	43.33	36.34	74	-----
	202	18.60	18.40	18.5	4,684.23	69.00	31.60	42.54	48	-----
	207	41.30	44.90	43.1	7,852.97	115.68	49.67	44.16	80	-----
	204	20.40	23.10	21.8	5,143.47	75.77	32.88	44.36	51	61 ± 3.37
	205	21.80	21.30	21.5	4,982.73	73.40	43.58	28.15	65	-----
Whole hay.	206	21.30	21.10	21.2	4,982.73	73.40	44.28	37.84	50	-----
502	201	25.90	27.70	26.8	8,193.60	204.66	57.38	89.08	61	-----
	202	18.40	20.90	19.7	7,488.04	187.04	55.58	62.63	70	-----
	207	42.20	43.10	42.6	9,490.92	237.06	65.75	130.67	52	59. ± 1.69
	204	21.80	23.60	22.7	7,488.04	187.04	55.19	81.93	58	-----
	205	22.70	24.90	23.8	7,488.04	187.04	49.74	81.46	56	-----
Leaves.	206	22.20	22.70	22.5	7,488.04	187.04	62.78	81.88	57	-----
503	201	25.60	23.50	24.6	5,551.50	197.53	40.16	124.07	41	-----
	202	21.10	20.00	20.6	5,551.50	197.53	42.31	113.36	46	-----
	207	40.60	39.50	40.1	8,327.25	296.30	57.34	191.67	42	-----
	204	21.50	21.80	21.7	5,551.50	197.53	38.56	133.06	37	41. ± .84
	205	22.20	23.60	22.9	5,551.50	197.53	42.87	122.82	39	-----
	206	20.90	21.90	21.4	5,551.50	197.53	37.97	123.31	42	-----

^a e = ewe and w = wether.^b No trial conducted.

TABLE 11.—*Biological values of proteins in stem, leaves, and whole hay of the first, second, and third cuttings of alfalfa when fed to 6 lambs as their sole ration during 10-day periods—Continued*

THIRD CUTTING

Ration and trial no	Lamb no.	Body weights			Dry matter feed intake	Nitrogen intake	Fecal nitrogen	Nitrogen in urine	Biological value of protein	Average value
		Initial	Final	Average						
Nitrogen free		<i>Kilo-grams</i>	<i>Kilo-grams</i>	<i>Kilo-grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>		
504	201	23.80	24.30	24 0	5,031.32	6.09	20.81	7.05		
	202	22.20	20.00	21 1	3,258.94	3.94	16.98	6.99		
	207	43.50	37.20	40.4	5,441.28	6.58	31.83	20.05		
	204	21.80	20.60	21 2	3,340.24	4.04	16.08	9.88		
	205	23.10	22.70	22 9	3,305.26	4.00	16.46	5.14		
	206	21.30	18.80	20 1	3,288.25	3.98	17.96	8.24		
Nitrogen free										
1000	1w	23.81	20.86	22.3	2,194.01	2.65	14.42	7.54		
	2w	24.72	23.81	24 3	2,291.38	2.77	15.17	8.36		
	3e	28.35	26.99	27.7	3,043.38	3.68	15.74	6.82		
	4w	29.03	26.99	28.0	2,584.40	3.13	22.95	7.27		
	5w	31.52	29.26	30 4	3,295.11	3.99	21.00	7.30		
	6w	33.79	29.94	31.9	3,004.82	3.61	20.37	15.79		
Stems.										
1001	1w	22.00	21.77	21.9	3,621.24	49.07	14.57	26.40	62	55. ± 1.58
	2w	25.40	24.49	24.9	4,403.51	59.72	25.08	29.17	66	
	3e	28.35	28.35	28.4	4,403.51	59.72	28.34	28.67	61	
	4w	27.67	27.22	27.1	4,403.51	59.72	43.03	20.64	70	
	5w	31.07	29.04	30.5	4,403.51	59.72	30.32	29.54	61	
	6w	30.84	29.49	30.2	4,403.51	59.72	28.27	35.22	64	
Whole hay										
1002	1w	20.87	22.68	21.8	5,618.64	158.04	43.43	74.31	55	54. ± .62
	2w	24.04	26.54	25.3	6,335.22	178.19	48.67	91.22	53	
	3e	26.99	29.48	28.2	6,506.23	183.00	47.98	91.17	51	
	4w	26.31	29.03	27.7	6,522.51	183.46	47.49	97.04	52	
	5w	29.48	31.98	30.7	6,644.66	186.00	48.15	88.00	55	
	6w	30.16	32.21	31.2	6,693.51	188.27	44.72	92.76	57	
Leaves										
1003	1w	23.59	23.13	23.4	4,311.19	148.19	35.21	92.64	39	42. ± .59
	2w	26.76	26.87	26.8	6,426.44	220.00	49.44	132.95	43	
	3e	30.16	30.39	30.3	6,855.43	235.61	50.91	137.21	42	
	4w	30.39	29.48	29.9	6,855.43	235.64	52.41	149.79	39	
	5w	34.02	33.31	33.7	6,855.43	235.64	56.24	132.85	44	
	6w	34.70	31.02	34.4	6,855.43	235.61	48.55	144.93	42	
Nitrogen free.										
1004	1w	24.04	23.13	23.6	3,042.30	3.68	16.25	8.56		
	2w	27.22	24.49	25.9	2,042.20	2.47	14.53	10.19		
	3e	33.79	31.52	32.7	3,882.50	4.70	21.78	9.47		
	4w	30.39	27.67	29.0	2,801.30	3.39	17.32	10.88		
	5w	32.66	30.84	31.8	3,334.80	4.04	18.78	8.57		
	6w	37.19	34.02	35.6	4,095.10	4.96	21.21	12.54		

TABLE 12.—*Biological values of proteins of stems, whole hay, and leaves of the first second, and third cuttings of alfalfa when fed to lambs*

Part of plant	First cutting		Second cutting		Third cutting		Weighted averages of all biological values
	Number of trials	Biological value of proteins	Number of trials	Biological value of proteins	Number of trials	Biological value of proteins	
Stems	5	67±4.55	6	61±3.77	6	65±1.58	64±1.93
Whole hay	6	41±2.27	6	59±1.09	6	54±0.82	51±1.55
Leaves	6	41±1.41	6	41±0.84	6	42±0.59	41±0.56

The level of protein intake for the stems, whole-hay, and leaf rations was approximately 8, 13, and 18.5 percent, while the fiber percentage in the same order was 40, 28, and 16. These differences in the chemical make-up of the rations should be kept in mind in comparing the biological values. Comparisons limited to the values for stems, leaves, or whole hay therefore appear to carry more weight than do those in which figures for stems are directly compared with those for leaves or the whole hay.

The somewhat lower biological values obtained for the stems of the second cutting, as compared with those for the first and the third were hardly in line with the biological value for the proteins of whole hay of the second cutting which was higher than that secured with the proteins of the whole hay of the first or third cuttings.

As pointed out (9, 10, 14, 43), the biological values of alfalfa proteins are relatively low, approximating those obtained by other workers with proteins of the corn grain.

CALCIUM AND PHOSPHORUS BALANCE STUDIES

Fifty-four complete calcium and the same number of phosphorus balances show further differences in the three cuttings (tables 13 and 14).

TABLE 13.—*Calcium and phosphorus balances of 18 lambs when fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa as their sole ration during 10-day periods*

FIRST CUTTING											
Part of plant and trial no	Lamb no	Calcium consumed	Calcium voided			Calcium balance	Phosphorus consumed	Phosphorus voided			Phosphorus balance
			Feces	Urine	Total			Feces	Urine	Total	
Stems											
102	1	43.82	52.15	0.10	52.25	-8.43	6.16	6.79	0.04	6.83	-0.67
	2	48.26	52.94	.16	53.10	-4.84	6.78	9.22	.05	9.27	-2.49
	3	48.26	60.79	.14	60.93	-12.67	6.78	6.41	.03	6.44	+3.89
103	4	49.92	47.19	.08	47.27	+2.65	7.01	7.86	.04	7.90	-4.89
	5	49.92	61.72	.12	61.84	-11.92	7.01	9.86	.03	9.89	-2.88
	6	49.92	61.27	.08	61.35	-11.43	7.01	8.59	.02	8.61	-1.60
Average		48.35	56.01	.11	56.12	-7.77	6.79	8.12	.04	8.16	-1.37
Whole hay:											
104	1	84.72	91.06	1.03	92.09	-7.37	7.93	5.81	.09	5.90	+2.03
	2	84.72	82.42	.38	82.80	+1.92	7.93	6.25	.09	6.34	+1.59
	3	84.72	83.77	.13	83.90	+ .82	7.93	6.77	.04	6.81	+1.12
105	4	76.11	76.21	.31	76.52	-.41	7.12	7.06	.08	7.14	-.02
	5	76.11	105.74	.14	105.88	-29.77	7.12	8.70	.05	8.75	-1.63
	6	76.11	79.81	.21	80.02	-3.91	7.12	7.33	.05	7.38	-.26
Average		80.42	86.50	.37	86.87	-6.45	7.52	7.14	.07	7.21	+1.31
Leaves											
106	1	170.44	153.16	.59	153.75	+16.69	10.83	8.96	.12	9.08	+1.75
	2	170.44	160.19	.46	160.65	+9.79	10.83	7.79	.12	7.91	+2.92
	3	170.44	166.42	.61	167.03	+3.41	10.83	11.40	.07	11.47	-.04
107	4	181.81	153.59	.29	153.88	+27.93	11.55	7.93	.08	8.01	+3.54
	5	180.38	75.17	.20	75.37	+105.01	11.46	8.43	.05	8.48	+2.96
	6	181.81	159.00	.40	159.40	+22.41	11.55	9.20	.18	9.38	+2.17
Average		175.89	144.59	.43	145.02	+30.87	11.18	8.95	.10	9.06	+2.12

TABLE 13.—Calcium and phosphorus balances of 18 lambs when fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa as their sole ration during 10-day periods—Continued

SECOND CUTTING

Part of plant and trial no.	Lamb no.	Calcium consumed	Calcium voided			Calcium balance	Phosphorus consumed	Phosphorus voided			Phosphorus balance
			Feces	Urine	Total			Feces	Urine	Total	
Stems.		Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
501	1	57.93	41.20	0.14	41.34	+16.59	13.95	9.36	0.21	9.57	+4.38
	2	40.61	34.42	.08	34.50	+6.11	9.78	9.05	.10	9.15	+0.63
	3	68.00	53.19	.05	53.24	+14.85	16.39	10.93	.18	11.11	+5.28
	4	44.59	31.43	.05	31.48	+13.11	10.74	7.04	.16	7.20	+3.54
	5	43.20	32.84	.02	32.86	+10.34	10.40	7.20	.09	7.29	+3.11
	6	43.20	28.55	.02	28.57	+14.63	10.40	6.12	.09	6.21	+4.19
Average		49.60	36.94	.06	37.00	12.60	11.94	8.28	.14	8.42	+3.52
Whole hay											
502	1	135.86	111.18	.37	111.55	+24.31	20.82	10.72	.21	10.93	+9.89
	2	124.17	108.27	.19	108.46	+15.71	19.02	11.62	.07	11.69	+7.33
	3	157.38	133.08	.23	133.31	+24.07	24.11	15.91	.25	16.16	+7.95
	4	124.17	109.14	.33	109.47	+14.70	19.02	12.74	.22	12.96	+6.06
	5	124.17	113.49	.08	113.57	+10.60	19.02	13.37	.16	13.53	+5.49
	6	124.17	112.96	.36	113.32	+10.85	19.02	13.12	.15	13.27	+5.75
Average		131.65	114.69	.26	114.95	+16.70	20.17	12.91	.18	13.09	+7.08
Leaves.											
503	1	141.28	112.00	.21	112.21	+29.07	17.34	3.60	1.48	5.08	+12.26
	2	141.28	120.97	.11	121.08	+20.20	17.34	3.25	.76	4.01	+13.33
	3	211.92	167.72	.15	167.87	+44.05	26.01	2.39	1.44	3.83	+22.18
	4	141.28	120.95	1.05	122.00	+19.28	17.34	7.77	1.21	1.98	+15.36
	5	141.28	123.55	.07	123.62	+17.66	17.34	2.46	.78	3.24	+14.10
	6	141.28	116.78	.24	117.02	+24.26	17.34	2.13	.54	2.67	+14.67
Average		153.05	127.00	.30	127.30	+25.75	18.78	2.43	1.04	3.47	+15.31

THIRD CUTTING

Stems											
1001	1	41.94	35.56	0.15	35.71	+6.23	3.87	3.93	0.10	4.03	-0.16
	2	51.00	51.76	.13	51.89	-.89	4.70	5.57	.13	5.70	-1.00
	3	51.00	55.79	.06	55.85	-4.85	4.70	5.14	.11	5.25	-.55
	4	51.00	52.68	.03	52.71	-1.71	4.70	4.74	.09	4.83	-.13
	5	51.00	55.64	.08	55.72	-4.72	4.70	4.98	.16	5.14	-.44
	6	51.00	50.75	.05	50.80	+0.20	4.70	6.17	.15	6.32	-1.62
Average		49.49	50.36	.08	50.45	-.96	4.56	5.09	.12	5.21	-.65
Whole hay											
1002	1	161.12	132.60	.30	132.90	+28.22	10.31	6.32	.10	6.42	+3.89
	2	181.66	151.75	.40	152.15	+29.51	11.67	7.71	.13	7.84	+3.83
	3	186.57	152.82	.22	153.04	+33.53	11.98	7.78	.13	7.91	+4.07
	4	187.03	157.93	.22	158.15	+28.88	12.02	6.59	.13	6.72	+5.30
	5	190.54	157.97	.36	158.33	+32.21	12.24	7.09	.16	7.85	+4.39
	6	191.94	153.55	.70	154.25	+37.69	12.33	7.36	.16	7.52	+4.81
Average		183.14	151.10	.37	151.47	+31.67	11.76	7.24	.14	7.38	+4.38
Leaves											
1003	1	146.24	138.11	3.18	141.29	+4.95	9.12	5.56	.85	6.41	+2.71
	2	217.90	191.62	3.29	194.91	+23.08	13.60	7.58	1.13	8.71	+4.89
	3	232.54	202.90	4.12	207.02	+25.52	14.51	7.70	.84	8.54	+5.97
	4	232.54	215.22	7.56	222.78	+9.76	14.51	9.45	1.58	11.03	+3.48
	5	232.54	218.82	4.21	223.03	+9.51	14.51	8.52	1.34	9.86	+4.65
	6	232.54	202.04	7.16	209.20	+23.34	14.51	7.44	1.44	8.88	+5.63
Average		215.73	194.78	4.92	199.70	+16.03	13.46	7.71	1.20	8.91	+4.55

TABLE 14.—Calcium and phosphorus balance data computed for stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to lambs

Cutting	Calcium consumed	Calcium voided			Calcium balance	Phosphorus consumed	Phosphorus voided			Phosphorus balance
		Feces	Urine	Total			Feces	Urine	Total	
	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
Stems:										
First	48.35	56.01	0.11	56.12	-7.77	6.79	8.12	0.04	8.16	-1.37
Second	49.60	36.94	.06	37.00	+12.60	11.94	8.28	.14	8.42	+3.52
Third	49.49	50.36	.08	50.45	-.96	4.56	5.09	.12	5.21	-.65
Average	49.15	47.77	.08	47.86	+1.29	7.76	7.16	.10	7.26	+ .50
Whole hay										
First	80.42	86.50	.37	86.87	-6.45	7.52	7.14	.07	7.21	+ .31
Second	131.65	114.69	.26	114.95	+16.70	20.17	12.91	.18	13.09	+7.08
Third	183.14	151.10	.37	151.47	+31.67	11.76	7.24	.14	7.38	+4.38
Average	131.74	117.43	.33	117.76	+13.98	13.15	9.10	.13	9.23	+3.92
Leaves:										
First	175.89	144.59	.43	145.02	+30.87	11.18	8.95	.10	9.06	+2.12
Second	153.05	127.00	.30	127.30	+25.75	18.78	2.45	1.04	3.47	+15.31
Third	215.73	194.78	4.92	199.70	+16.03	13.46	7.71	1.20	8.91	+4.55
Average	181.56	155.46	1.88	157.34	+24.22	14.47	6.36	.78	7.15	+7.32

The stems of the second cutting were better sources of calcium and phosphorus than those of the first and third cuttings. The intake of calcium per lamb for the 10-day periods varied from 40.6 to 68.1 g.

The phosphorus balances for the second-cutting stem ration were all positive. The 10-day intake of phosphorus per lamb varied from 9.8 to 16.4 g, and its storage from 0.6 to 5.3 g.

In contrast with these figures are those for the stem ration of first-cutting hay. The calcium intake per lamb was more or less uniform, ranging from 43.8 to 49.9 g per lamb for the 10-day period. However, every lamb except no. 4 lost from 4.8 to 12.7 g of calcium. One lamb gained 2.7 g. The lambs consumed from 6.2 to 7 g. per head of phosphorus in the 10-days, and every lamb but one showed losses. These losses varied from 0.7 to 2.9 g., while a gain of only 0.3 g. is shown for lamb 3.

Results with stems of the third cutting showed 2 positive and 4 negative calcium balances, and all negative phosphorus balances. The stems, because of their bulk and low percentage of both calcium and phosphorus, when fed as the sole feed, resulted in negative balances in most cases, the ration of stems of the second-cutting hay being an exception.

Whole-hay rations showed the second and third cuttings to be good sources of both calcium and phosphorus, as evidenced by strongly positive balances. In contrast were the results when lambs were fed first-cutting hay. The calcium and phosphorus intake was lower than for the second and third cuttings, and all the lambs but two showed losses of calcium.

The leaf rations supplied most calcium. They were also highest in phosphorus in the first and third cuttings. The second-cutting leaf rations on the average, however, were lower in phosphorus. All the leaf rations produced positive calcium balances, and the data show that the leaves are excellent sources of calcium.

The leaf rations were also apparently good sources of phosphorus. When the first-cutting leaves were fed, all but one lamb stored some

phosphorus, the quantities varying from 1.8 to 3.5 g. per lamb for the 10-day period. One lamb lost 0.6 g. of phosphorus. The leaves of the second cutting excelled all others as sources of phosphorus, and third-cutting leaves ranked second in this respect.

The average results in table 14 show that a lamb fed alfalfa stems for 10 days will consume approximately 49 g of calcium, of which slightly less than 1.3 g will be retained by the body. These results, however, may be misleading, because losses occurred when first- and third-cutting stems were fed, and the excellent storage of calcium when second-cutting stems were fed more than offset the losses. The same was true of phosphorus. The average intake of phosphorus per lamb for the 10-day period was approximately 7.8 g, 0.5 g being stored. Again the second-cutting stems resulted in excellent storage of phosphorus, so as to offset the negative results secured with rations of first- and third-cutting stems when the balances were averaged.

An average of all data for three cuttings of whole hay shows that a lamb consumed 131.7 g of calcium, of which 14 g were retained. When first-cutting hay was fed, losses resulted. The feeding of third-cutting hay resulted in the most favorable storage of calcium. The calcium retention of 14 g per lamb as an average for all lambs was accompanied by a storage of 5 g of phosphorus. Actual losses of calcium occurred when first-cutting hay was fed. The second cutting was the best source of phosphorus.

The results as summarized for leaf rations show an intake of 181.6 g of calcium for the 10-day period, accompanied by a storage of 24 g. All balances were positive, and strangely enough the feeding of leaves of first-cutting hay resulted in the most favorable storage of calcium.

The average intake of phosphorus in the leaf rations for this period was 14.5 g, of which 7 g was stored. Second-cutting leaves produced the most favorable balances.

Table 14 shows strikingly the value of leaves as sources of available calcium and phosphorus. The whole hay ranked second, but the average results with first-cutting whole hay showed actual losses of calcium and phosphorus.

The results with stems show negative balances for all but the second-cutting stems. Throughout the entire study the results with second-cutting stems and leaves show them especially to be excellent sources of calcium and phosphorus. Most of the phosphorus was excreted by way of the intestines, as was shown by Ross et al. (31).

SUMMARY AND CONCLUSIONS

In 10 experiments reported in this paper, 67 to 83 percent of the total protein of the alfalfa plant was contained in the leaves, as well as 71 to 85 percent of the calcium, and 46 to 79 percent of the phosphorus.

The leaves exerted a marked diuretic effect on lambs in the decreasing order of first, second and third cuttings.

The trend was toward an almost equal partition of excretory nitrogen between feces and urine when stems were fed; with the exception of the second cutting, approximately 67 percent of the nitrogen was found in the urine with whole-hay rations; 70 to 78 percent of the nitrogen excreted when leaf rations were fed was found in the urine.

The coefficients of apparent digestibility, averages for 18 lambs and of 3 cuttings were as follows: (1) For the stems, dry matter 46.6, crude protein 51.1, crude fiber 39.0, fat 47.9, and nitrogen-free extract 58.9; (2) for the whole hay, dry matter 56.3, crude protein 69.7, crude fiber 40.0, fat 24.0, and nitrogen-free extract 71.0; (3) for the leaves, dry matter 66.3, crude protein 77.4, crude fiber 55.5, fat 29.7, and nitrogen-free extract 75.9. Coefficients for the stems, whole hay, and leaves of each of the three cuttings are reported separately.

A given weight of leaves was almost 3.57 times as efficient as the same weight of stems in supplying digestible protein, and the leaves were less bulky.

The percentage of digestible nutrients in the stems, whole hay, and leaves of alfalfa, average for three cuttings and with 18 lambs were as follows: Stems, 4.17 percent digestible crude protein and 41.55 percent total digestible nutrients; whole hay, 9.77 percent digestible crude protein and 48.43 percent total digestible nutrients; leaves, 14.87 percent digestible crude protein, and 57.82 percent total digestible nutrients.

When alfalfa stems (average of all three cuttings) were fed, 5.2 percent of the nitrogen was stored; when whole hay was fed, 17 percent was retained; when leaves were fed, 16.3 percent was stored. The slightly lower retention when leaves were fed was possibly due to an excessive supply of nitrogen above the optimum necessary for maintenance and growth and for the small requirements incident to slight fattening and wool growth.

The weighted averages of biological values of the proteins from the three cuttings as determined with 18 growing lambs were as follows: (1) Stems, 64; (2) whole hay, 51; (3) leaves, 41. The variation in values are attributed to the differences in protein concentration of the stem, leaf, and whole-hay rations.

Lambs fed stems of the three cuttings consumed during a 10-day period a total of 49.15 g of calcium and 7.76 g of phosphorus. The balances show a retention of 1.29 g of calcium and 0.50 g of phosphorus.

When whole alfalfa hay was fed, the lambs consumed on the average 131.74 g of calcium each 10 days, and of this they retained 13.98 g. The phosphorus intake was 13.15 g and the retention 3.92 g.

The leaf rations (all three cuttings) contained 181.56 g of calcium per lamb for the 10-day period of which 24.22 g were retained. The phosphorus content was 14.47 g and the retention 7.32 g.

Complete data for each lamb fed stems, leaves, and whole hay of the three cuttings are included. They support the conclusion that alfalfa leaves are important sources of calcium and phosphorus, in spite of the unfavorable ratio in which these are found.

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EFFECT OF THE RIPENING PROCESS OF CHEESE ON THE NUTRITIVE VALUE OF THE PROTEIN OF MILK CURD¹

By JESSIE R. BEADLES, *assistant in animal nutrition*, J. H. QUISENBERRY, *assistant in animal genetics*, F. I. NAKAMURA, *assistant in animal nutrition*, and H. H. MITCHELL, *professor and chief in animal nutrition, Illinois Agricultural Experiment Station*

INTRODUCTION

The ripening of cheese is essentially a hydrolysis of the paracasein in the rennet curd through the agency of proteases (5).² During this proteolysis the concentration of water-soluble nitrogen, as also of nonprotein and amino nitrogen, in the cheese increases rather evenly with time (6). Insofar as these changes predominate in the ripening of cheese, there is no reason to suspect that the nutritive value of the rennet-curd protein would be affected. However, the products of this proteolysis, perhaps largely through the influence of bacteria, undergo certain secondary reactions, involving oxidation, reduction, deamination, and decarboxylation (2, 15). The products of these reactions may differ widely from the products of simple proteolysis with respect to their value in animal nutrition, though their occurrence in cheeses ripened mainly by other agencies than bacteria may be of minor importance. Nelson (10) reported the presence of indole and phenols in Limburger cheese, while Camembert cheese contained a trace of indole but no phenols.

No attempt appears to have been made to determine whether and to what extent the nutritive value of the protein of the rennet curd has been affected by the chemical changes incident to ripening. The present and the increasing importance of cheese in the American diet warrants a thorough study of this subject. This paper represents a contribution to it, involving a series of controlled-feeding experiments and metabolism studies on rats.

MATERIAL STUDIED

The cheeses studied were American Cheddar, Roquefort, Swiss, and Limburger. The latter three varieties were furnished by the Kraft-Phenix Cheese Corporation of Chicago. The American Cheddar cheese, and the fresh rennet curd from cow's milk, with which all cheeses were compared, were supplied by the Department of Dairy Husbandry of the University of Illinois.

The fresh cheese was ground, mixed, and sampled for chemical analysis. It was then dried at a low temperature before a blower, ground, and extracted with ether to remove practically all the fat. The dried residue was then analyzed for nitrogen and fat. The ether-soluble material removed from each cheese was also analyzed for nitrogen.

The chemical composition of the fresh cheese is given in table 1. The percentage of nitrogen varied from 9.75 (Roquefort) to 13.25

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² Reference is made by number (*italic*) to Literature Cited, p. 18.

(milk curd) in the dried and extracted cheese and in the dried milk curd used in the preparation of the experimental rations. The fat content of these preparations was low (about 0.2 percent). The nitrogen content of the ether-soluble material removed from the cheese was very small, ranging from 0.0037 to 0.031 percent. It is evident that only an insignificant portion of the cheese nitrogen was removed from the experimental samples by ether extraction.

TABLE 1.—Chemical composition of the fresh cheese

Variety	Water	Protein (N×6.38)	Fat	Ash	Calcium	Phos- phorus	Gross energy per gram
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Calories</i>
American Cheddar.							
Sample 1	35.33	25.26	28.30	4.04	0.845	0.534	4.46
Sample 2	31.81	24.56	31.17	3.77			4.90
Swiss	35.70	32.03	17.63	4.05	1.05	.728	4.25
Roquefort							
Sample 1	36.84	22.14	33.35	7.38			
Sample 2	40.33	20.03	32.08	6.89	.550	.424	4.06
Limburger							
Sample 1	48.00	21.25	26.37	3.68	.656	.418	3.83
Sample 2	57.46	21.56	28.65	3.59	.524	.368	4.17

In a sample of Cheddar cheese and a sample of Limburger cheese the writers attempted, by the use of Richmond's method (13, p. 231), to differentiate the primary products of ripening (precipitated as basic copper compounds) and the secondary products of ripening (not so precipitated) in the water-soluble constituents of the cheese. In Cheddar cheese it was found that 29 percent of the total nitrogen was water soluble, and of the water-soluble nitrogen, 62.6 percent was not precipitated by copper sulphate (CuSO_4) and sodium hydroxide (NaOH). In Limburger cheese, these percentages were, respectively, 34.6 and 56.6.

EXPERIMENTAL PROCEDURE

The first comparisons of cheese protein and milk-curd protein were made by the paired-feeding method, rats being used as subjects. Eight or nine pairs of rats were used in each comparison. Pair mates were always of the same sex, approximately of the same initial weight, and in the large majority of cases they were litter mates. The rats were confined in individual wire cages of the usual design, and the food consumption of pair mates was equalized, so that the only difference in their treatment was the difference in composition of the rations compared. In each comparison of cheese with milk curd, one rat in each pair received the cheese ration and the other the milk-curd ration. These rations contained the same nonprotein ingredients and practically the same percentage of nitrogen, and they had very nearly the same gross energy value as determined by the bomb calorimeter. The nitrogen content of all rations was brought close to 1.28 percent, equivalent to 8 percent of crude protein. This level of protein will permit considerable growth if the protein possesses a high biological value and yet will not promote maximum growth with the best of proteins, a point of great importance in the technic of protein comparison.

All the experimental rations contained 4 or 4.5 percent, either of the original Osborne-Mendel salt mixture (12) or, later, of the modified

mixture proposed by Wesson (14), 8 percent of clarified butterfat, 2 percent of cod-liver oil, 10 percent of sucrose, 1 percent of sodium chloride, and 1 percent of dried yeast. Each ration also contained enough of the dried extracted cheese or the dried rennet curd (approximately 10 percent) to provide 1.28 percent of nitrogen, and enough starch to make up 100 percent. The rations were apparently all deficient in vitamin B₁, since shortly after the start of each experiment it was necessary to give a few drops of tiki-tiki extract to each rat to stimulate a failing appetite. In this, as in every other respect, pair mates were treated exactly alike.

The rats were weighed for 3 consecutive days at the beginning and the end of the feeding period, the average of each group of three weights constituting the initial or the final weight of the rat. Weekly weights were also taken throughout the experiments which continued for 7 or 8 weeks.

In the process of equating the food intakes of pair mates, the amount of food offered to the rats in each pair was increased as long as both rats cleaned up their dishes and was reduced whenever food residues remained in either of the dishes at the end of the day. Notes were kept of the number of times that each rat refused food throughout the experiment. A comparison of these food refusals between pair mates affords information of the comparative avidity with which the experimental rations were being consumed, or possibly of their comparative palatability. Such information is of interest in testing the frequently expressed (or implied) opinion that the better balanced of any two rations will always be consumed in the greater amount in a feeding experiment in which unrestricted access to the feed is permitted.

At the end of each experiment, the rats used therein were killed with ether, and their body lengths from mouth to anus were measured. Differences in the stage of growth between pair mates would presumably be the only gross factor determining differences in body length, while other gross factors, such as differences in the deposition of fat, would affect body weight.

EXPERIMENTAL RESULTS

GROWTH-PROMOTING VALUE OF THE PROTEIN OF CHEESE AND OF RENNET MILK CURD

The results of these paired-feeding tests are summarized in tables 2, 3, 4, and 5, while a statistical summary is given in table 6. In all tests but that of the Limburger cheese the results favored the cheese as a source of protein over the milk curd. However, in only one comparison the Swiss cheese with milk curd are the differences between pair mates significant. In this case the rat receiving the cheese ration gained faster than its pair mate in 7 of the 8 pairs, while of the weekly comparisons of gains 39.5 favored the cheese ration and only 16.5 favored the milk-curd ration. When the total gains are analyzed by Student's method (1) the mean difference in gain between pair mates is 7.25 grams (favoring the cheese ration), the standard deviation of differences is 7.15 grams, and the ratio z is 1.01. For $n=8$ and $z=1.01$ the probability that the mean difference was the result of a fortuitous combination of factors common to both groups of rats is only 0.016, and is thus so small (equivalent to 1 of 62 events) that it may be neglected. It may

be concluded, therefore, that the differences observed between pair mates in rate of gain in weight was produced by the difference in the character of the dietary proteins. However, no consistent differences between pair mates were noted with respect to body length (4 comparisons favoring one ration and 4 the other), so that there may be a legitimate objection to interpreting the difference in rate of gain on the two rations as a difference in rate of growth.

The Roquefort cheese also promoted greater gains in weight than the milk curd in most of the experimental pairs. Analysis of this experiment, gives $M = +5.75$, $s = 10.35$, $z = 0.56$, and $P = 0.11$. This probability is too large to be neglected, so that the possibility that the outcome was a result of chance cannot be removed.

In all comparisons the cheese rations were considerably less frequently refused; in other words, they were the most palatable, whether or not they promoted the most rapid gains in weight.

TABLE 2.—Comparison of the growth-promoting value of the protein of Cheddar cheese and of rennet milk curd by the paired-feeding method during a feeding period of 60 days

Item	Pair 1, males		Pair 2, females		Pair 3, females		Pair 4, females		Pair 5, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams	151	154	150	145	143	151	127	134	139	142
Initial weight.....do	85	90	91	87	81	86	48	48	49	51
Gain.....do	66	64	59	58	62	65	79	86	90	91
Body length.....centimeters	19.4	19.9	19.0	18.7	18.6	18.9	18.3	18.1	18.3	18.3
Total food.....grams	515	515	512	512	520	520	503	503	521	521

Item	Pair 6, females		Pair 7, males		Pair 8, males		Pair 9, males	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams	111	118	118	119	121	120	115	116
Initial weight.....do	35	37	36	37	38	39	37	37
Gain.....do	76	81	82	82	83	81	78	79
Body length.....centimeters	17.4	17.7	18.2	18.6	18.2	18.3	17.7	17.9
Total food.....grams	458	458	448	448	429	429	440	440

TABLE 3.—Comparison of the growth-promoting value of the protein of Roquefort cheese and of rennet milk curd by the paired-feeding method during a feeding period of 56 days

Item	Pair 1, males		Pair 2, males		Pair 3, males		Pair 4, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams	111	128	122	133	97	115	126	112
Initial weight.....do	67	66	71	70	63	63	61	63
Gain.....do	44	62	51	63	34	52	65	49
Body length.....centimeters	17.9	18.2	18.3	18.8	17.7	17.7	17.8	17.6
Total food.....grams	447	447	481	481	379	380	422	425

Item	Pair 5, males		Pair 6, males		Pair 7, females		Pair 8, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams	128	133	118	126	126	126	133	134
Initial weight.....do	65	66	63	64	61	61	65	63
Gain.....do	63	67	55	62	65	65	68	71
Body length.....centimeters	18.2	18.1	18.0	18.4	18.0	17.8	17.8	18.3
Total food.....grams	408	408	402	402	446	448	451	451

TABLE 4.—*Comparison of the growth-promoting value of the protein of Swiss cheese and of rennet milk curd by the paired-feeding method during a feeding period of 50 days*

Item	Pair 1, males		Pair 2, males		Pair 3, females		Pair 4, males	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams	152	153	129	143	133	133	123	132
Initial weight.....do	65	68	64	56	66	63	42	39
Total gain.....do	87	85	65	87	67	70	81	93
Body length.....centimeters	19.6	19.6	18.8	18.6	19.0	18.8	18.9	18.8
Total food.....grams	477	477	423	423	430	430	421	421

Item	Pair 5, females		Pair 6, females		Pair 7, males		Pair 8, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams	121	121	124	129	123	127	111	122
Initial weight.....do	38	39	39	39	38	38	38	37
Gain.....do	83	85	85	90	85	89	73	85
Body length.....centimeters	17.8	18.0	18.3	18.3	18.7	18.8	17.3	17.6
Total food.....grams	410	410	450	450	415	415	400	400

TABLE 5.—*Comparison of the growth-promoting value of the protein of Limburger cheese and of rennet milk curd by the paired-feeding method during a feeding period of 50 days*

Item	Pair 1, males		Pair 2, males		Pair 3, females		Pair 4, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams	130	138	153	145	136	137	148	145
Initial weight.....do	49	53	60	57	51	54	56	54
Gain.....do	81	85	93	88	85	83	92	91
Body length.....centimeters	19.1	19.0	19.7	19.6	18.9	18.5	19.2	18.8
Total food.....grams	444	444	457	457	459	459	512	512

Item	Pair 5, males		Pair 6, males		Pair 7, females		Pair 8, males	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams	116	115	121	125	121	119	100	102
Initial weight.....do	39	41	43	42	42	43	37	36
Gain.....do	77	74	78	83	79	76	63	66
Body length.....centimeters	18.3	18.2	18.5	18.4	17.8	18.3	17.2	17.7
Total food.....grams	373	373	433	433	397	397	350	350

TABLE 6.—*Statistical data on the paired-feeding experiment*

Item	Milk curd	Cheddar cheese	Milk curd	Roquefort cheese	Milk curd	Swiss cheese	Milk curd	Limburger cheese
Number of food refusals.....	103	33	105	49	29	12	34	4
Comparison of weekly gains of pair mates ¹	33.5	38.5	28	31	16.5	39.5	29.5	26.5
Comparison of total gains of pair mates ¹	3.5	5.5	1.5	6.5	1	7	5	3
Comparison of body length of pair mates ¹	2.5	6.5	3.5	4.5	4	4	6	2

¹ The figures in these comparisons represent the number of comparisons favoring either the rat receiving milk curd or the rat receiving cheese. In the case of equal measurements (gains in weight or body lengths) each pair mate is credited with 0.5.

COMPARATIVE DIGESTIBILITY OF CHEESE PROTEIN AND MILK-CURD PROTEIN

The comparative digestibility of cheese and milk-curd protein was determined in two of the paired-feeding experiments, those involving Roquefort and Limburger cheese. The collection periods were of 7 days' duration, throughout which the intake of food was kept the same. The coefficients of apparent digestibility for pair mates receiving the same amounts of food are summarized in table 7. For all pairs of rats the milk-curd protein was digested more completely. In one series of pairs the average coefficient for milk-curd protein was 89.65 and that for Roquefort-cheese protein 87.28. In the other series of pairs the average coefficient for the milk-curd protein was 88.78 and that for Limburger-cheese protein 87.22. Furthermore, in a later test (table 9), it was found that Swiss-cheese protein was rather consistently less digestible than milk-curd protein, the average digestion coefficients being, respectively, 89.12 and 90.53.

TABLE 7.—Coefficients of apparent digestibility of the protein of rennet milk curd and of Roquefort and Limburger cheese for the different pairs of rats

Pair no.	Milk curd	Roquefort cheese	Milk curd	Limburger cheese	Pair no.	Milk curd	Roquefort cheese	Milk curd	Limburger cheese
1.	89.2	86.3	88.4	88.2	6.	88.9	86.3	89.0	86.2
2.	89.7	87.8	87.4	86.8	7.	89.8	86.7	87.8	86.8
3.	89.0	87.3	88.6	86.9	8.	89.2	88.0	90.5	89.9
4.	91.2	86.3	89.6	87.2					
5.	90.1	89.5	88.9	85.8	Average....	89.65	87.28	88.78	87.22

It thus appears that cheese protein supports a more rapid gain in weight in albino rats than does milk-curd protein, although it is clearly, if only slightly, less digestible. This somewhat anomalous situation is explainable on three possible grounds: (1) The voluntary activity of the rats on the cheese ration was enough less than that of those on the milk-curd ration to reduce the dietary energy available for gains in weight to an extent that compensated (or more than compensated) for the less complete digestibility of the cheese protein; or (2) the biological value of cheese protein is greater than that of milk-curd protein, and enough greater partly to offset the less complete digestibility, and in one case (Swiss cheese) to more than offset it; or (3) the gains in weight produced on the cheese rations contain less protein and more fat than those produced on the milk-curd rations.

Concerning the first possible explanation, the writers have no information to offer at all. Nevertheless, it is certainly well to keep in mind that the results of feeding experiments may be modified, or even determined by a differential effect on the voluntary activity of the rats of the two rations being compared. For example, if one ration, possibly because of a poorer balance of nutrients in it, depresses voluntary muscular activity, it may promote as great a rate of gain in body weight as a better balanced ration, even when consumed in no greater amounts, because a greater proportion of it will be available for increase in weight. The effect of qualitatively different rations upon the activity of experimental animals in nutrition laboratories is practically a virgin field of study, although in the interest of the refinement of the technic of feeding experiments, it is a field that needs careful investigation.

RELATIVE BIOLOGICAL VALUES OF MILK-CURD AND CHEESE PROTEINS

To determine the relative biological values of the proteins of milk curd and of cheese, a study was made of Swiss cheese, which had given clear indications in the paired-feeding experiments of a greater capacity to promote gains in weight than had milk curd. Ten rats were used in these experiments. The plan of the experiment, as well as the technic of the metabolism work, was essentially the same as that developed in this laboratory and described elsewhere (3). The 10 rats were used as 2 groups of 5 each, and the plan of feeding was as follows:

Rats 1 to 5, inclusive	Rats 6 to 10, inclusive
Period 1. Standardizing ration	Standardizing ration
Period 2. Milk-curd ration	Swiss-cheese ration
Period 3. Swiss-cheese ration	Milk-curd ration
Period 4. Milk-curd ration	Swiss-cheese ration
Period 5. Standardizing ration	Standardizing ration

The experimental rations had the composition shown in table 8.

TABLE 8.—Composition of rations used in studying the relative biological values of the proteins of milk curd and of Swiss cheese

Constituent	Standard- izing ration	Milk- curd ration	Swiss- cheese ration
	Percent	Percent	Percent
Dried, fat-free whole egg.....	5 47		
Dried rennet-milk curd.....			10 60
Dried, fat-free Swiss cheese.....		10 13	
Modified Osborne-Mendel salts.....	4 5	4 5	4 5
Clarified butterfat.....	8	8	8
Cod-liver oil.....	2	2	2
Sucrose.....	10	10	10
NaCl.....	1	1	1
Harris yeast vitamin powder.....	0 3	0 3	0 3
Starch.....	66 73	62 07	61.6
Pigment.....	2	2	2
Protein content.....	4 37	8 39	8.34

In order to improve the consumption of the experimental rations, 3 drops of tiki-tiki extract (containing 9.7 mg of nitrogen) were given to each rat daily when signs of a diminished appetite became apparent.

In these metabolism experiments feces markers were used to differentiate the feces of the experimental periods from those of the preceding and following preliminary (or transition) periods. On the first day of an experimental period the ration contained 2 percent of ferric oxide (Fe_2O_3), which colored the first day's feces a brilliant red; for the remaining 6 days of the period 2 percent of barium sulphate (BaSO_4) replaced the ferric oxide, giving to the feces a lighter than normal appearance; then again on the first day following the termination of the experimental period, the ration containing 2 percent of ferric oxide was given.

The purpose of the initial and final standardizing periods was to determine the excretion of body nitrogen in feces (the so-called "metabolic nitrogen") and in urine (the endogenous nitrogen), the former being dependent upon the dry matter consumed and the latter upon the body weight of the rat. The average excretion of metabolic nitrogen in the feces was 1.2 mg and 1.37 mg per gram of food consumed in the two periods. The endogenous nitrogen output

in the urine per 100 g body weight averaged 22.0 mg in the first standardizing period and 20.7 mg in the second. These factors differ somewhat in the two periods for each rat, and in computing the body's contribution to urine and feces in the intermediate experimental periods, they are assumed to change in a linear fashion from the initial to the final value determined.

TABLE 9.—*Nitrogen metabolism of rats on milk-curd and Swiss-cheese rations*

[Results expressed on the daily basis]

RATS 1 TO 5						
Ration and rat no.	Initial body weight	Final body weight	Food intake	Nitrogen intake	Nitrogen in feces	Nitrogen in urine
	Grams	Grams	Grams	Multi- grams	Milli- grams	Milli- grams
Standardizing ration						
1	48	50	8.1		8.8	11.1
2	47	53	7.3		8.1	10.7
3	50	60	8.3		8.9	12.7
4	45	53	7.2		8.1	11.1
5	45	50	6.8		7.3	11.8
Milk-curd ration						
1	66	86	10.0	138.3	12.8	45.8
2	60	75	8.5	123.7	11.0	48.5
3	64	81	9.2	129.6	12.6	52.6
4	58	72	7.9	118.3	11.4	45.7
5	58	66	5.8	112.9	8.7	41.6
Swiss-cheese ration						
1	90	106	10.0	138.1	15.4	55.7
2	78	91	8.7	126.6	12.9	54.7
3	87	97	9.4	128.5	14.8	60.3
4	74	89	8.8	131.3	13.4	50.5
5	64	77	8.2	112.8	15.0	46.5
Milk-curd ration.						
1	117	133	11.5	159.1	15.4	67.1
2	99	112	9.9	136.7	(12.5)	59.7
3	104	122	11.0	149.5	(13.7)	61.9
4	97	108	9.3	131.8	12.4	60.0
5	85	96	8.5	122.2	10.9	47.1
Standardizing ration:						
1	137	150	11.4		15.0	22.6
2	119	123	9.6		12.9	26.7
3	126	140	12.0		14.0	26.1
4	112	122	10.7		13.4	23.4
5	100	110	9.9		12.5	22.7
RATS 6 TO 10						
Standardizing ration.						
6	50	56	6.7		7.7	10.4
7	46	52	5.8		6.8	10.7
8	49	58	7.3		8.1	11.4
9	49	56	7.1		8.1	12.2
10	47	55	7.1		7.9	11.0
Swiss-cheese ration.						
6	59	68	6.6	90.0	10.1	34.3
7	57	69	6.4	89.9	8.7	30.0
8	65	80	8.9	118.7	11.8	43.6
9	63	77	8.7	118.9	12.2	41.2
10	60	71	8.2	110.1	11.3	45.1
Milk-curd ration:						
6	75	87	8.0	115.7	12.3	49.5
7	74	87	8.1	115.2	10.7	45.6
8	88	102	9.3	131.8	13.2	58.8
9	85	99	9.2	130.8	13.2	55.6
10	76	89	8.7	124.7	12.7	53.6
Swiss-cheese ration:						
6	92	102	8.1	117.9	13.5	55.8
7	93	103	7.2	109.8	12.2	41.9
8	105	117	9.7	133.5	13.5	57.4
9	104	116	9.9	135.3	15.2	64.7
10	95	104	9.3	128.6	14.3	65.6
Standardizing ration:						
6	108	112	8.2		12.6	19.7
7	110	117	8.8		11.7	20.2
8	124	132	10.8		14.4	24.3
9	121	129	11.3		17.1	30.9
10	111	111	7.8		12.6	31.7

All the nitrogen-metabolism data of this experiment are assembled in table 9, while the digestion coefficients and biological values computed from them are summarized in tables 10 and 11. To conserve space in the tables the intermediate calculations are omitted, the method of calculation having been fully explained in earlier publications from this laboratory.

TABLE 10.—*Coefficients of apparent and true digestibility of the protein of Swiss cheese and of rennet milk curd*¹

Rat no	Apparent digestibility				True digestibility			
	Milk-curd protein		Swiss-cheese protein		Milk-curd protein		Swiss-cheese protein	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1	90.7	90.3	88.6		99.0	99.5	97.5	
2	91.1	90.9	89.8		99.3	100.0	98.4	
3	90.3		88.4		98.2	99.3	96.8	
4	90.4	90.6	89.8		98.3	99.3	97.8	
5	92.3	91.1	86.7		98.3	99.7	95.3	
6	89.3		88.8	88.5	98.8		98.0	98.4
7	90.7		90.3	88.9	99.5		98.9	97.3
8	90.0		90.1	89.8	98.6		98.8	99.0
9	89.9		89.7	88.8	99.3		98.7	99.2
10	89.8		89.7	88.9	99.3		98.9	99.6
Average	90.53		89.12		99.09		98.17	

¹ For rats 1 to 5, 2 digestion trials were run on the milk-curd ration, while for rats 6 to 10, 2 digestion trials were run on the Swiss-cheese ration.

TABLE 11.—*Biological values of the protein of Swiss cheese and of rennet milk curd*

Rat no	Milk-curd protein		Swiss-cheese protein		Rat no	Milk-curd protein		Swiss-cheese protein	
	Trial 1	Trial 2	Trial 1	Trial 2		Trial 1	Trial 2	Trial 1	Trial 2
1	77	71	71		7	74		81	78
2	72	73	71		8	70		76	73
3	71	73	67		9	74		79	72
4	73	71	74		10	74		73	70
5	76	78	72						
6	70		75	67	Average	73.1		73.3	

An illustration of the calculation of a biological value for protein is the following: Rat no. 6 in period 2 consumed 6.59 g daily of the Swiss-cheese ration, containing 90 mg of nitrogen. The average daily excretion of nitrogen in the feces was 10.1 mg, of which 8.3 mg (1.26×6.59) was estimated to be of body origin. Hence, the fecal nitrogen of food origin was equal to $10.1 - 8.3 = 1.8$ mg. The absorbed food nitrogen therefore is equal to $90 - 1.8 = 88.2$ mg. The urinary nitrogen for the period averaged 34.3 mg daily, the sum of 12.5 mg of endogenous nitrogen (19.2×65 , the average body weight of the rat for the period) and 21.8 mg of food nitrogen unutilized in metabolism. Of the nitrogen absorbed, $88.2 - 21.8 = 66.4$ mg were retained in the body for both maintenance and growth. The biological value is this figure expressed as a percentage of the absorbed nitrogen, i.e. $(66.4 \div 88.2) \times 100 = 75$.

The digestion coefficients given in table 10 again reveal a lower digestibility of cheese nitrogen than of milk-curd nitrogen. The average coefficients of apparent digestibility are 89.12 and 90.53, respectively. If the fecal nitrogen is reduced by the amount of

metabolic nitrogen estimated to be present in the feces, it is possible to compute coefficients of true digestibility, which average 98.17 and 99.09, respectively, the advantage again resting with the milk-curd protein.

However, the biological values computed for the two protein sources were very nearly the same, averaging 73.1 for milk-curd protein and 73.3 for Swiss-cheese protein (table 11).

These determinations of the biological values of the protein of milk curd and of Swiss cheese indicate that the good showing of the cheese proteins in the paired-feeding experiments, in spite of a distinctly lower digestibility, cannot be explained on the basis of a higher utilization of their absorbed nitrogen in the anabolic chemical reactions of maintenance and growth. The remaining possibility is that the explanation is to be found in the composition of the gains in weight produced on the two types of rations, one containing milk curd as its main source of protein and one containing cheese as its predominant source of protein.

NITROGEN AND ENERGY CONTENT OF PAIRED RATS ON MILK-CURD AND LIMBURGER-CHEESE RATIONS

In order to investigate this possibility, the 16 rats used in the paired-feeding comparison of milk curd and Limburger cheese (table 5) were analyzed after removal of the gastrointestinal tract and contents. The feeding experiment had shown no advantage for either sort of protein in the promotion of gains in weight but did show a clear advantage of the milk-curd protein over the cheese protein in digestibility (table 7). The nitrogen contents of the rat carcasses were determined by the Kjeldahl method, and their gross energy values (heats of combustion) by the Parr oxygen bomb calorimeter. The results of these determinations are given in table 12.

TABLE 12. *Nitrogen and energy contents of paired rats on milk-curd and Limburger-cheese rations*

Pair no.	Protein in ration	Body weight of rat minus alimentary tract	Nitrogen content		Energy content	
			Percent	Grams	Per gram	Total
		<i>Grams</i>			<i>Calories</i>	<i>Calories</i>
1	Milk curd.....	123	2.57	3.16	2.24	276
	Cheese.....	130	2.32	3.02	2.43	315
2	Milk curd.....	144	2.69	3.87	2.63	365
	Cheese.....	135	2.74	3.70	2.34	316
3	Milk curd.....	129	2.70	3.48	2.26	292
	Cheese.....	129	2.55	3.29	2.33	301
4	Milk curd.....	140	2.68	3.75	2.22	311
	Cheese.....	137	2.52	3.45	2.42	332
5	Milk curd.....	109	2.69	2.93	1.89	206
	Cheese.....	109	2.68	2.92	2.21	241
6	Milk curd.....	113	2.82	3.19	1.84	208
	Cheese.....	117	2.36	2.76	2.11	247
7	Milk curd.....	114	2.65	3.02	2.00	228
	Cheese.....	112	2.39	2.68	2.51	281
8	Milk curd.....	94	2.90	2.73	2.06	194
	Cheese.....	95	2.67	2.54	2.09	199

An analysis according to the Student method of the differences between pair mates with respect to their nitrogen and energy contents is given in table 13.

TABLE 13.—*Analysis according to the Student method of the differences between pair mates with respect to nitrogen and energy contents*

Item	Nitrogen content		Energy content	
	Percent	Grams	Per gram	Total
Mean of differences, M^1	+0.182	+0.221	<i>Calories</i> —0.175	<i>Calories</i> —19.0
Standard deviation, s	.148	.122	.196	29.9
Ratio $z = M/s$	1.23	1.81	.89	.64
Probability, P	.0071	.001	.025	.008

¹ The differences between pair mates were given a plus sign when the value for the rat on the milk-curd ration was the larger, and a negative sign when it was the smaller.

In every pair of rats but one, the percentage of nitrogen was higher for the rat on the milk-curd diet, and in all eight pairs the weight of nitrogen was higher. The probabilities that these paired differences were determined only by chance are so low (i.e., 0.0071 and 0.001, respectively) that they may be neglected. It seems certain, therefore, that the difference in the rations produced the difference in nitrogen content of the rats, the milk-curd ration promoting, to a greater extent, the deposition of nitrogen during growth.

The values for the energy contents of the rats presents a different picture, though not so convincing. In every pair but one the energy content of the rat receiving the Limburger-cheese ration, whether expressed in calories per gram of rat or in total calories, was greater than that of the rat raised on the milk-curd ration. The probabilities that chance alone could have produced these results may not be small enough to disregard entirely, although with respect to the gross energy per gram chance would be expected to produce such a one-sided outcome once in about 40 times. In this case at least it may be said with practical certainty that the difference in ration was responsible, the Limburger-cheese ration producing a carcass with the higher gross-energy content presumably because of a greater content of fat.

TABLE 14.—*Estimated gains in nitrogen and gross energy of paired rats on milk-curd and Limburger-cheese rations*

Pair no.	Protein of ration	Body weights minus intestinal tract		Estimated initial content of rats in—		Observed final content of rats in—		Gains in—	
		Initial ¹	Final	Nitrogen	Energy	Nitrogen	Energy	Nitrogen	Energy
		Grams	Grams	Grams	Calories	Grams	Calories	Grams	Calories
1	Milk curd.....	42	123	1.14	63	3.16	276	2.02	213
	Cheese.....	46	130	1.25	69	3.02	315	1.77	246
2	Milk curd.....	53	144	1.44	80	3.87	365	2.43	285
	Cheese.....	50	135	1.36	75	3.70	316	2.34	241
3	Milk curd.....	44	129	1.20	66	3.48	292	2.28	226
	Cheese.....	47	129	1.28	71	3.29	301	2.01	230
4	Milk curd.....	49	140	1.33	74	3.75	311	2.42	237
	Cheese.....	47	137	1.28	71	3.45	332	2.17	261
5	Milk curd.....	33	109	.90	50	2.93	206	2.03	156
	Cheese.....	35	109	.95	53	2.92	241	1.97	188
6	Milk curd.....	37	113	1.01	56	3.19	208	2.18	152
	Cheese.....	36	117	.98	54	2.76	247	1.78	193
7	Milk curd.....	36	114	.98	54	3.02	228	2.04	174
	Cheese.....	37	112	1.01	56	2.68	281	1.67	225
8	Milk curd.....	31	94	.84	47	2.73	194	1.89	147
	Cheese.....	30	95	.82	45	2.64	199	1.72	154

¹ Estimated.

In order to eliminate in these comparisons the results of variable initial contents of nitrogen and energy among the paired rats, the gains in nitrogen and in energy were computed from the observed final contents and from estimates of the initial contents based on the initial weights. The initial weights of the rats were corrected to the weights minus the intestinal tract by the use of Donaldson's tables (4). The remainder of the carcass was then assumed to contain 2.72 percent of nitrogen and 1.505 calories of gross energy per gram on the basis of the results of Mitchell and Carman (9). The computation of the gains of nitrogen and energy is given in table 14.

The differences between pair mates in the storage of nitrogen averaged 232 mg in favor, in each pair, of the milk-curd ration. The standard deviation of differences was 113 mg, and the probability that this outcome is a result of chance is only 0.0005, clearly negligible. The differences between pair mates in energy storage averaged 18.5 calories, and in all pairs but one favored the Limburger-cheese ration. However, the standard deviation of differences is 27.9 calories and the desired probability is 0.063. These results are therefore merely highly suggestive of a ration effect.

It appears that the less digestible Limburger-cheese nitrogen promoted a smaller storage of nitrogen in the paired-feeding experiment than the same intake of milk-curd nitrogen. Nevertheless, the gains in weight were just as large, due in all probability to a greater deposition of fat in the rats on the cheese ration.

TABLE 15.—*Estimation of the biological values of the protein of milk curd and Limburger cheese when fed to paired rats*

Pair no	Protein of ration	Average body weight	Total intake of digestible nitrogen	Total endogenous nitrogen	Nitrogen stored in tissues	Total nitrogen utilized	Biological value of nitrogen
		Grams	Grams	Grams	Grams	Grams	Percent
1	Milk curd	90.4	5.25	0.99	2.02	3.01	57
	Cheese	96.5	5.24	1.06	1.77	2.83	54
2	Milk curd	106.0	5.34	1.17	2.43	3.60	67
	Cheese	100.6	5.30	1.11	2.34	3.45	65
3	Milk curd	95.7	5.44	1.05	2.28	3.33	61
	Cheese	96.0	5.34	1.06	2.01	3.07	57
4	Milk curd	104.1	6.13	1.15	2.42	3.57	58
	Cheese	100.5	5.96	1.11	2.17	3.28	55
5	Milk curd	79.5	4.45	.87	2.03	2.90	65
	Cheese	78.5	4.29	.86	1.97	2.83	66
6	Milk curd	84.5	5.16	.93	2.18	3.11	60
	Cheese	84.0	5.00	.92	1.78	2.70	54
7	Milk curd	81.9	4.67	.90	2.04	2.94	63
	Cheese	81.0	4.62	.89	1.67	2.56	55
8	Milk curd	67.7	4.25	.75	1.89	2.64	62
	Cheese	68.5	4.23	.75	1.72	2.47	58

It is possible from the data at hand to determine more or less certainly whether the smaller storage of nitrogen on the cheese ration was entirely accounted for by the smaller digestibility of the cheese nitrogen. In table 15 will be found computations similar in import to those leading to the determination of biological values from the data of metabolism experiments. The only explanation necessary relates to the computation of the endogenous nitrogen. The values given for this item cover the entire period of the feeding experiment. They are based upon the average weight of the rat for the feeding

period and an average value, taken from metabolism experiments, of 22 mg of endogenous nitrogen per day per 100 g of body weight. It should be said of the biological values given in the last column that they may be less than those obtained in a short metabolism experiment if, during the prolonged feeding period, the rat has shed appreciable amounts of hair and scurf. In such case the nitrogen stored in the tissues is really greater than the difference between the initial and final nitrogen content of the rat.

From the table it is evident that in all pairs but one the computed biological value of the dietary protein is smaller for the Limburger cheese than for the milk curd, the averages being, respectively, 58 and 62. The average difference is 3.63 and the standard deviation of differences 2.49. The probability that chance alone would account for the paired differences is only 0.0031. It seems, therefore, that the biological value of Limburger-cheese protein is somewhat less (about 6 percent) than that of its mother substance, the milk-curd protein. Such a difference was not detected in metabolism experiments affording a comparison of the protein of Swiss cheese and of milk curd (table 11), though this fact cannot be considered inconsistent with the finding for Limburger cheese because of the more extensive ripening to which the latter cheese was evidently subjected.

If the protein of milk curd, casein, has suffered a deterioration in nutritive value during the conversion of the curd into Limburger cheese, then it may be expected that the amino acid deficiency of the casein would be altered. It has been demonstrated that casein is deficient in the sulphur-containing amino acid, cystine (11). If, during the ripening process, cystine is destroyed more rapidly than other amino acids indispensable in nutrition, then the cystine deficiency of the cheese protein will be greater than the cystine deficiency of casein, so that a greater improvement in its biological value will result when it is supplemented with an excess of cystine. On the other hand, if some other indispensable amino acid is destroyed more rapidly than the cystine, then the resulting cheese protein will possess a smaller deficiency of cystine, or even no cystine deficiency at all.

CYSTINE DEFICIENCIES OF MILK-CURD AND LIMBURGER-CHEESE PROTEINS

In measuring the cystine deficiencies of the protein of milk curd and the protein of Limburger cheese, the paired-feeding technic was modified. The ordinary technic as used in the preceding experiments is for some purposes open to the criticism that the better of two rations being compared is in a sense being penalized, because the rats receiving it are growing at the faster rate and are correspondingly increasing their maintenance requirements over those of their pair mates. Hence, of the equal intakes of food, a smaller proportion of the better ration will be available for growth over and above the maintenance requirements. Osborne and Mendel (11, p. 9) have discussed the same situation and have concluded: "The only strict basis for comparison is afforded by experiments in which the animals receive the same amount of food during the same period of time and make the same gain in weight."

This principle was incorporated into the paired-feeding technic, as illustrated by the milk-curd experiment. Several kilograms of a milk-curd ration containing 8 percent of protein, and otherwise

similar in make-up to the rations used in the preceding experiments, were prepared, thoroughly mixed in an electric mixer, and divided into two equal portions. One portion was used unchanged, while to the other portion was added 0.3 percent of cystine, equivalent to 3.75 percent of the protein. This proportion of cystine is more than enough to supplement the casein. The two rations were then fed to a series of pairs of rats in the ordinary way until differences in weight developed between pair mates. Then the rat in each pair that received the cystine diet was fed a combination of this diet and of a low-nitrogen diet similar to it except that it contained no cystine and that starch was substituted for the dried milk curd. The total amount of the two rations fed to the rats receiving cystine was kept the same as the amount of food consumed by their pair mates on the unsupplemented milk-curd ration, while the proportion between the amounts of the two combined rations was so adjusted that the gains in weight of pair mates were equalized as closely as possible. Thus, as the rats on the cystine ration increased in weight beyond their pair mates, they were given a greater proportion of the low-nitrogen diet, and vice versa. From 3 to 5 drops of tiki-tiki extract were given daily to each rat in order to maintain the appetite.

TABLE 16.—*Supplementing effect of cystine on the protein of rennet milk curd during a feeding period of 49 days when fed to paired rats*

Item	Pair 1, females		Pair 2, females		Pair 3, females		Pair 4, females	
	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine
Final weight..... grams	122	121	129	128	115	121	128	125
Initial weight..... do.	46	44	50	50	49	49	50	50
Gain..... do.	76	77	79	78	66	72	78	75
Body length..... centimeters	18.4	18.3	18.4	18.4	18.3	18.6	18.2	18.1
Food intake..... grams	443	443	466	466	428	428	447	447
Nitrogen intake..... do.	5.71	4.41	6.01	4.23	5.53	3.86	5.77	4.34
Ratio of nitrogen intakes.....	1.29	1	1.42	1	1.43	1	1.33	1

Item	Pair 5, females		Pair 6, females		Pair 7, males		Pair 8, females	
	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine
Final weight..... grams	138	133	126	120	153	152	138	142
Initial weight..... do.	48	46	49	45	57	55	53	56
Gain..... do.	90	87	77	75	96	97	85	86
Body length..... centimeters	18.8	18.9	18.0	18.2	19.5	19.4	18.9	19.1
Food intake..... grams	447	447	451	451	500	500	488	488
Nitrogen intake..... do.	5.77	5.15	5.82	4.50	6.45	4.96	6.30	5.22
Ratio of nitrogen intakes.....	1.12	1	1.29	1	1.30	1	1.21	1

The results of this type of an experiment on milk-curd protein are given in table 16. On the same intakes of food the gains in weight of pair mates were kept very nearly the same (except in one pair in which the difference amounted to 6 g) simply by varying the nitrogen intake. A considerably smaller amount of nitrogen from the mixture of casein and cystine promoted the same gains in weight. From the ratios between the nitrogen intakes of pair mates, given in the last line of the table, it appears that 1 g of nitrogen from the casein-cystine mixture

produced the same nutritive effect as 1.12 to 1.43 g of nitrogen (averaging 1.30 g) from the unsupplemented casein. Taking the biological value of casein as 73, that of the supplemented casein would be $73 \times 1.3 = 95$,³ on the reasonable assumption that cystine did not affect the digestibility of the casein. Mitchell (8) has reported metabolism data on a rat, indicating a biological value of 75 for casein, and a value of 92 for casein fully supplemented with cystine.

Three pairs of rats in this experiment, nos. 1, 7, and 8, were analyzed at the end of the feeding period to determine whether the composition of the carcasses had been affected by the difference in dietary treatment. Only the nitrogen and energy contents of the empty carcasses were determined. The results are given in table 17.

TABLE 17.—*Nitrogen and energy contents of empty carcasses of three pairs of rats used in experiments for which data are recorded in table 16*

Pair no.	Dietary protein	Nitrogen content	Energy content per gram
		Percent	Calories
1	(Milk curd.....	3.34	2.07
	(Milk curd+cystine.....	3.30	2.18
7	(Milk curd.....	3.03	2.30
	(Milk curd+cystine.....	3.02	2.64
8	(Milk curd.....	3.18	2.31
	(Milk curd+cystine.....	3.28	2.22

The differences between pair mates were slight and did not show any consistency, so that it may be concluded that the similar gains of pair mates signified similar nitrogen retention. For these six rats computations of the biological value of the dietary protein were made, according to the method used in a preceding experiment (tables 14 and 15), with the results given in table 18. The unsupplemented milk-curd protein exhibited in this test biological values of 73, 73, and 70, which are of the same magnitude as those determined by the nitrogen-balance studies (table 11). The supplemented casein gave biological values of 93, 95, and 88, averaging 92. The results of the feeding experiment led to an estimate of 95.

TABLE 18.—*Estimation of the biological values of the protein of milk curd with and without a cystine supplement when fed to paired rats*

Pair no.	Supplement	Total intake of digestible nitrogen	Total endogenous nitrogen	Total nitrogen stored in tissues	Total nitrogen utilized	Biological value
		Grams	Grams	Grams	Grams	Percent
1	(None.....	5.15	0.95	2.83	3.78	73
	(Cystine.....	3.98	.95	2.75	3.70	93
7	(None.....	5.80	1.12	3.09	4.21	73
	(Cystine.....	4.43	1.12	3.09	4.21	95
8	(None.....	5.66	1.06	2.91	3.97	70
	(Cystine.....	4.71	1.08	3.06	4.14	88

³ It must be admitted that part of this increase in the biological value of casein accompanying the addition of cystine may be due to the fact that the supplemented casein was fed at a lower percentage of the diet than the unsupplemented. This in itself would tend to increase the biological value, although all proteins do not show this effect (7).

The Limburger cheese was tested by the same methods applied to a study of the cystine deficiency of milk-cured protein. Eight pairs of rats were used in a preliminary test and were carried for 2 weeks on equalized intakes of the supplemented and unsupplemented rations containing equal percentages of nitrogen. The purpose of this test was to determine whether the protein of Limburger cheese was deficient in cystine. At the end of 2 weeks of feeding such a deficiency was clearly established. In seven pairs, the greater gains were made by the rat receiving the cystine-supplemented diet, while in one pair the gains were equal. The average difference in gain between pair mates was 3.50 g, and the standard deviation of differences was 2.46 g. The probability that the average difference was the result merely of a fortuitous combination of factors common to both rats of a pair is only 0.0036, certainly small enough to be negligible.

TABLE 19.—*Supplementing effect of cystine on the protein of Limburger cheese during a feeding period of 45 days when fed to paired rats*

Item	Pair 1, females		Pair 2, males		Pair 3, females		Pair 4, males	
	No cystine	Cys-tine	No cystine	Cys-tine	No cystine	Cys-tine	No cystine	Cys-tine
Final weight..... grams.....	85	86	98	97	95	98	110	108
Initial weight..... do.....	42	42	40	39	44	47	47	45
Gain..... do.....	43	44	58	58	51	51	63	63
Body length..... centimeters.....	16 5	16 8	17 0	17 2	16 9	17 0	17 4	17 6
Food intake..... grams.....	305	305	290	290	292	292	282	282
Nitrogen intake..... do.....	4 14	3 61	3 93	3 47	3 97	3 67	3 83	3 20
Ratio of nitrogen intakes.....	1 15	1	1 13	1	1 08	1	1 20	1

Item	Pair 5, males		Pair 6, males		Pair 7, females		Pair 8, females		Pair 9, females	
	No cystine	Cys-tine	No cystine	Cys-tine	No cystine	Cys-tine	No cystine	Cys-tine	No cystine	Cys-tine
Final weight..... grams.....	87	87	100	100	112	112	100	99	102	101
Initial weight..... do.....	38	39	43	42	48	48	46	43	32	32
Gain..... do.....	49	48	57	58	64	64	54	56	70	69
Body length..... centimeters.....	17 8	17 8	17 3	17 7	17 8	18 3	17 3	17 4	17 4	17 5
Food intake..... grams.....	255	255	296	296	375	375	319	319	328	328
Nitrogen intake..... do.....	3 46	3 33	4 02	3 57	5 08	4 51	4 33	3 75	4 45	3 95
Ratio of nitrogen intakes.....	1 04	1	1 13	1	1 13	1	1 15	1	1 13	1

A cystine deficiency in the protein of Limburger cheese having been established, the extent of this deficiency was quantitatively assessed by the modified paired-feeding technic. Nine pairs of rats were used in the experiment. The results obtained are summarized in table 19. A very close equalization of the gains of pair mates was secured in this test, but a relatively greater proportion of the cheese-cystine nitrogen was required than of the casein-cystine nitrogen in the preceding test. The ratios of nitrogen intakes from the unsupplemented and the supplemented cheese rations ranged from 1.04 to 1.20 to 1, and averaged 1.13 to 1. If the biological value of the Limburger-cheese protein may be taken as 6 percent less than that of casein (Table 11), namely 69, then the addition of cystine increased it only 13 percent, giving a value of 78. This is appreciably less than the value of 92 to 95 given by milk-curd protein plus cystine. It may be concluded, therefore,

that the ripening of the curd in the manufacture of Limburger cheese lowered the biological value of the protein by reactions involving the destruction of cystine to some extent, but to a greater extent the destruction of some one or more of the other amino acids indispensable in animal nutrition. We may infer that cystine has been involved to some extent in these reactions both because it is still the limiting deficiency of the cheese protein, and because the biological value of the cheese protein appears to be somewhat less than that of the curd protein. We may infer that other indispensable amino acids are also involved, because the improvement in biological value induced by the addition of cystine is considerably less than the improvement noted in the case of casein. In other words, a second limiting amino acid deficiency is manifest in the supplemented cheese protein at a lower biological value than in the supplemented curd protein.

DISCUSSION

The most general and clear-cut finding of the feeding and metabolism experiments reported in this paper on four varieties of cheese (Cheddar, Roquefort, Swiss, and Limburger) was that in the ripening process to which milk curd is subjected in the manufacture of cheese, the digestibility of the protein of the curd is depressed 1 or 2 percent. However, the capacity of the cheese protein to promote gains in weight was not lowered. In fact, in the case of Swiss cheese, its gain-promoting capacity was definitely, though slightly, increased over that of milk curd. In metabolism experiments on 10 rats no differences in the biological values of rennet curd and of Swiss cheese could be distinguished. Hence, the depressed digestibility of the protein of this cheese could be reconciled with its increased capacity to promote gains in weight in young rats, most probably on the assumption that the chemical composition of the weight increases on cheese protein showed less protein and more fat than those produced by milk-curd protein.

This assumption was tested for Limburger cheese, the protein of which was found to be as efficient as milk-curd protein in promoting gains in weight, within the limits of accuracy of the paired-feeding method, although it also was distinctly less digestible. By analyzing the carcasses of the rats at the conclusion of the paired-feeding tests, and from average analyses of rats at the initial weight, computing the nitrogen and gross energy content of the gains in weight produced, it was clearly shown that the assumption was correct. It was also shown that in the case of Limburger cheese the biological value of the protein was slightly depressed, about 6 percent, below that of the casein of milk curd. The contrast in this respect between this protein and that of Swiss cheese is probably related to the more extensive ripening which the Limburger cheese undergoes.

The fact that equal gains in weight put on in the same periods on the same amounts of two rations may differ appreciably in composition, indicates that a gram gain in weight in experimental rats possesses a variable nutritive significance, although in the current interpretation of feeding experiments on rats, less well controlled than were these, the nutritive significance of gains in weight are assumed to vary only with their size. This experiment on Limburger

cheese confirms the conclusion of Mitchell and Carman (9, p. 410), expressed 8 years ago:

Therefore, the gain in weight of an animal, no matter how carefully experimental conditions may be prescribed, is not a definite measure of the nutritive effect of the ration fed, but is subject to considerable variation due to the operation of unknown factors.

It proves beyond doubt that the exact interpretation of feeding experiments, no matter how well controlled they may be, requires an investigation of the chemical composition of the carcasses produced by the experimental feeding.

The analyses of the carcasses of experimental rats in these studies have permitted the computation of the biological value of dietary protein by another method than that based on the metabolism data secured over a short period. The former biological values may be less than the latter on account of the loss of hair and scurf during a long feeding period, but in one case, involving a study of milk-curd protein, the two computations led to practically identical results.

The nutritive value of Limburger-cheese protein, and presumably of that of the proteins of other cheeses in which the chemical changes of ripening are no more extensive, is limited by a deficiency of cystine, in a similar manner, though not to so great an extent, as the paracasein of milk curd. In studying the quantitative aspects of the cystine deficiency of cheese and milk curd, a modification of the paired-feeding method was used, involving a principle enunciated by Osborne and Mendel in one of their earlier series of feeding experiments, namely, that in the comparison of the growth-promoting values of proteins the experimental animals should receive "the same amount of food during the same period of time and make the same gain in weight", the only variable being the intake of protein.

CONCLUSIONS

The digestibility of the protein of Roquefort, Swiss, and Limburger cheese is from 1 to 2 percent less than that of rennet milk curd.

The biological value of the protein of Swiss cheese is practically the same as that of rennet curd, i.e., 73, while the biological value of the protein of Limburger cheese is about 6 percent less.

In Limburger cheese, and presumably in all cheeses in which ripening has proceeded no further, cystine is the amino acid limiting its biological utilization, as is true of casein itself. However, the biological value of the protein of Limburger cheese is not increased as much by cystine supplementation as is the biological value of casein.

Cystine destruction is involved in the ripening reactions in the preparation of Limburger cheese, as is also one other, at least, of the amino acids indispensable in animal nutrition.

The accurate interpretation of the gains in weight of animals secured in feeding experiments, no matter how well controlled, requires an investigation of their nutrient content.

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THE EFFECT OF EGG PRODUCTS ON THE WHIPPING PROPERTIES OF ICE-CREAM MIXES MADE FROM BUTTER AND DRIED SKIM MILK¹

By C. C. WATTS, *fellow in dairy husbandry*, and C. D. DAHLE, *professor of dairy manufactures, Department of Dairy Husbandry and Bacteriology, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Several investigators (11, 12, 18)² have demonstrated that the whipping properties of ice-cream mixes can be markedly improved by the addition of egg yolk to the mix, and considerable research (2, 3, 4, 5, 21, 22) has been conducted to determine why certain products produce the beneficial effects noted. At present, however, these researches have not shown conclusively why egg yolk exerts so decided an effect upon the whipping properties of the ice-cream mixture.

The experiments reported in this paper were made to determine the effect produced by the addition of various egg products to ice-cream mixes made with unsalted butter, dried skim milk, and water.

REVIEW OF LITERATURE

Button (2, 3) separated dried yolk into yolk protein, egg oil, lecithin, and cholesterol. He considered that the egg oil and the lecithin cause the slow initial rise of overrun when egg yolk is used in the mix. The improvement in whipping properties he attributed to the egg-yolk protein. He found egg albumin to be detrimental to the whipping qualities of the mix; it exhibited no stabilizing properties and injured both the texture and the flavor of the ice cream. Dried egg yolk and dried whole egg did not contribute a stabilizing effect but did improve the texture, particularly in low-solid mixes. Less time was required to obtain 100 percent overrun when dried egg yolk and dried whole eggs were added to low-solid mixes. Flavors due to egg products were more pronounced in low-solid mixes than in high-solid mixes.

Tracy and Ruehe (22) and Caulfield and Martin (4) found that the addition of egg or soybean lecithin to ice-cream mixes did not improve their whipping properties and that soybean lecithin acted as a deterrent to overrun. Dahle (5) also reported the failure of soybean lecithin in amounts ranging from 0.1 to 0.4 percent to exert any beneficial effect on the whipping properties of a mix.

Martin (10), after studying the effect of certain acetone and ether extracts from egg yolk, reached no conclusion as to why egg yolk was beneficial to the whipping properties of an ice-cream mix.

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² Reference is made by number (*italic*) to Literature Cited, p. 976.

Sommer and Horrall (21) were unable to secure any improvement in the whipping properties of ice-cream mixes containing egg lecithin, egg oil, or egg-yolk protein, either separately or combined.

Richardson (20, p. 435) gives the composition of egg yolk, according to Gobley, as follows:

	Percent		Percent
Glycerophosphoric acid.....	1.2	Cholesterol.....	0.4
Vitellin.....	15.8	Fats.....	20.3
Nuclein.....	1.5	Coloring matters.....	.5
Cerebrin.....	.3	Salts.....	1.0
Lecithin.....	7.2	Water.....	51.8

METHOD OF PROCEDURE

All the ice-cream mixes were made from Extra grade, spray-process, dried skim milk and fresh, good quality unsalted butter, except certain control mixes, in which fresh 40 percent cream was used as the source of fat. Cane sugar was employed as the sweetening agent. The gelatin tested 220 Bloom grams. The egg lecithin and soybean lecithin used were obtained from recognized sources.

Sixty-pound mixes, made in 10-gallon cans, had the following composition: 12 percent fat, 11 percent serum solids, 15 percent sugar, 0.25 to 0.37 percent gelatin, and 38.25 percent total solids. All mixes were pasteurized at 150° F. for 30 minutes. The mixes were viscolized at 2,000 pounds pressure at 150°, a single-stage valve being used unless otherwise noted. The mixes were cooled quickly over a surface cooler to approximately 40° F. and aged 20 to 22 hours at 40°. For freezing the mixes a 40-quart brine freezer was used. The brine temperatures ranged from -5° to -10° F. but were held constant during a series of freezings. The temperature of the ice cream and the percentage of overrun were recorded at 1-minute intervals until 100 percent overrun had been obtained. An ammeter device was used to aid the freezer operator in turning off the brine. The freezer was cooled by freezing a preliminary batch of regular stock mix before the experimental mixes were frozen. Samples of ice cream were drawn for scoring at exactly 100 percent overrun except in the few cases mentioned where this overrun was not obtainable.

After hardening 3 to 5 days at -10° F. the ice cream was scored independently by three judges. It will be noted that the scores reported in the tables contain decimals. These are due to the averaging of the scores rendered by the different judges.

EFFECT OF DIFFERENT PRODUCTS ON WHIPPING PROPERTIES OF THE MIX AND QUALITY OF THE ICE CREAM

DRIED EGG YOLK

The effect of dried egg yolk upon the whipping properties of a mix was first studied. Control mixes were made with cream or unsalted butter, dried skim milk, and water; experimental mixes were made with cream or unsalted butter, dried skim milk, and water to which was added 0.5 percent of dried egg yolk. Viscolization in these experiments was accomplished with a two-stage valve at the pasteurization temperature. The data obtained are presented in table 1.

TABLE 1.—Effect of egg yolk on the whipping properties of the mix and quality of the ice cream

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3-5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
	<i>Minutes</i>				
Cream; dried skim milk; water	8 15	43.58	23.50	5	72.08
Cream; dried skim milk; water; 0.5 percent dried egg yolk	7 50	43.27	23.50	5	71.77
Butter; dried skim milk; water	10 25	43.00	23.50	5	71.50
Butter; dried skim milk; water; 0.5 percent dried egg yolk	7 65	42.84	23.50	5	71.34

The results in table 1 show that the addition of 0.5 percent of dried egg yolk to the butter-dried skim milk mixture effected a minimum saving of 2.6 minutes in the time required to obtain 100 percent overrun. In additional experiments herein reported, as much as 8 minutes saving was noted when 0.5 percent of dried egg yolk was added to butter-dried skim milk mixes. The same amount of egg yolk added to mixes made with cream and dried skim milk resulted in a saving of a little over half a minute. Thus the egg yolk exerted a much more beneficial effect upon the whipping properties of butter-dried skim milk mixes than upon those of cream-dried skim milk mixes.

Ice cream containing cream and egg yolk scored higher than that made from butter and egg yolk.

DRIED DOMESTIC V. CHINESE EGG YOLK; DRIED WHOLE EGG

A study was made of the effect of domestic and Chinese dried egg yolk and of domestic dried whole egg upon the whipping properties of mixes made with butter, dried skim milk, and water. Five tenths percent of the various egg products was added to the experimental mixes. The results are given in table 2.

TABLE 2.—Effect of whole egg and various kinds of egg yolk or dried whole egg, 0.5 percent in all cases, on the whipping properties of the mix and quality of the ice cream

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3-5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
	<i>Minutes</i>				
Butter; dried skim-milk; water	16.50	35.00	23.66	5	63.66
Butter; dried skim milk; water; dried Chinese egg yolk	8.75	35.00	24.20	5	64.20
Butter; dried skim milk; water; dried domestic egg yolk, brand A	9.00	35.33	24.50	5	64.83
Butter; dried skim milk; water; dried domestic egg yolk, brand B	8.50	33.33	24.50	5	62.83
Butter; dried skim milk; water; dried whole egg	9.20	33.33	24.08	5	62.41

An examination of table 2 reveals little difference in the effect of domestic and Chinese egg yolk upon the whipping properties of mixes made with butter and dried skim milk. Both types of yolk materially shortened the time required to obtain the desired yield. The maximum saving in time thus obtained was 8 minutes.

The dried whole egg also exerted a beneficial effect upon the whipping properties of the mix, but not to the same extent as did the dried egg yolk.

As the butter was somewhat old the scores of the ice cream in these experiments were all low.

SWEETENED FROZEN YOLK AND DRIED YOLK

The effect of sweetened frozen egg yolk as compared with that of dried egg yolk was studied. Mixes were made with butter, dried skim milk, and water to which 1 percent of previously frozen egg yolk containing 10 percent of sucrose was added. Other mixes were made of the same ingredients except that instead of frozen egg yolk 0.5 percent of dried egg yolk was added. The results are presented in table 3.

TABLE 3.—*Effect of sweetened frozen egg yolk and dried egg yolk on the whipping properties of the mix and quality of the ice cream*

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3-5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
Butter; dried skim milk; water	Minutes 13	43 00	23 50	5	71 50
Butter, dried skim milk; water, 1 percent sweetened frozen egg yolk *	9	43 00	23.50	5	71 50
Butter; dried skim milk; water; 0.5 percent dried egg yolk *	6	42.66	23.50	5	71.16

* The amount of egg-yolk solids in the sweetened frozen yolk was equivalent to the egg-yolk solids in the dried egg yolk

Table 3 shows that dried egg yolk had a more beneficial effect on the whipping properties of the mix than did the frozen egg yolk. A saving of 7 minutes in the time required to obtain the desired yield was noted in the case of the mixes containing dried egg yolk, whereas only 4 minutes saving was obtained in the case of the mixes containing sweetened frozen yolk.

Since the actual amount of egg-yolk solids in the sweetened frozen egg yolk was equivalent to the egg-yolk solids in the dried egg yolk, the poorer results obtained with sweetened frozen egg yolk may have been due to the previous freezing of the sweetened yolk or to the presence of sugar in the frozen yolk.

Urbain and Miller (23) consider that the lecithin of egg yolk separates out on freezing but that this can be partially prevented by adding certain sugars before freezing the yolk. Price (19) noted that the whipping properties of mixes containing frozen egg yolk were inferior to those of mixes containing frozen egg yolk which had previously been frozen with invert sugar. He attributed the difference

to a lesser separation of lecithin in the sweetened yolk during the freezing of the yolk.

The score of the ice cream made with sweetened frozen yolk, as shown in table 3, was the same as the score of the control ice cream, but the ice cream containing the dried yolk scored a trifle less than the control.

CERTAIN EGG-YOLK CONSTITUENTS

In order to study the effect of the various compounds making up egg yolk upon the whipping properties of butter-dried skim milk mixes, dried egg yolk was separated into certain component parts. The egg-yolk salts were obtained by two methods. According to the first method, 4 pounds of dried egg yolk was dialyzed in large collodion bags suspended in distilled water. The dialyzate was condensed in a Pyrex-glass condensing apparatus under 20 to 25 inches of vacuum. An aliquot of the condensate was used in an ice-cream mix. The second method consisted of the ultrafiltration of 0.3 pound of dried egg yolk dissolved in 2 liters of distilled water, which was allowed to stand for a time at 40° F. This amount of egg yolk is equal to approximately 0.5 percent of the weight of the ice-cream mixes made. The ultrafilters consisted of three no. 50 Whatman filter papers previously impregnated with one layer of collodion. These papers were placed in the bottom of a large Büchner funnel and the outer edges were cemented to the sides of the funnel by a thick layer of paraffin. An extra heavy suction flask was used and approximately 25 inches of vacuum were employed in the filtration. Before the egg-yolk liquid was added to the ultrafilter it was filtered through a coarse filter paper in a 40° F. refrigerated room. This first filtrate was subsequently ultrafiltered. The ultrafiltrate was free from colloids and gave strong tests for chlorides. The entire ultrafiltrate was added to a mix made with butter, dried skim milk, and water, allowance being made for the added water of the ultrafiltrate. The residual egg yolk containing the proteins, fat, and phospholipids was used in another ice-cream mix. It was thought that this latter method of securing the egg-yolk salts was less likely to cause alterations in the mix. When the dialysis method was used no preservative was employed and a slight bacterial decomposition occurred during the rather long dialyzing period.

The vitellin and lecithin of the egg yolk were separated by methods given by Richardson (20). The lecithin was not separated from the fats, cholesterol, and glycerophosphoric acid but was left with these compounds in order that their combined effect might be observed, since the action of lecithin in purer form was to be studied later.

Mixes were prepared from butter, dried skim milk, and water, and the various egg products were added. Control mixes were made from cream or butter and dried skim milk. The results of these experiments are shown in table 4.

Table 4 indicates that of the various fractions separated from egg yolk, vitellin, which composes the greater portion of the egg-yolk proteins, was detrimental to the whipping properties of the mix. The egg-yolk salts exerted a somewhat beneficial effect. The portion of the egg yolk containing the phospholipids—the portion in which lecithin is most abundant—was likewise beneficial. The part of the yolk containing the salts and proteins in combination was only slightly so.

TABLE 4.—*Effect of certain constituents of egg yolk on the whipping properties of the mix and quality of the ice cream*

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3-5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
	<i>Minutes</i>				
Cream; dried skim milk; water	9 85	43 33	24 16	5	72.49
Butter; dried skim milk; water	13 75	41 33	24.33	5	70.66
Cream; dried skim milk; water; 0.5 percent dried egg yolk	8.00	43 33	24.50	5	72.83
Butter; dried skim milk; water; 0.5 percent dried egg yolk	8 40	40 16	24.50	5	69.66
Butter; dried skim milk; water; egg-yolk salts	12 15	37 33	24.16	5	66.49
Butter; dried skim milk; water; vitellin from egg yolk	14.00	36 66	24 00	5	65.66
Butter; dried skim milk; water; ether-soluble extract from egg yolk ^a	12 50	35 00	24 00	5	64.00
Butter; dried skim milk; water; ether-extracted egg-yolk residue ^b	13 00	35 33	24 00	5	64.33

^a This extract contained the fats, cholesterol, lecithin, and glyce-ro-phosphoric acid

^b The residue from the extracted egg yolk mentioned in footnote 1 was composed chiefly of the egg-yolk proteins and salts

Sommer (21, p. 309-310) states:

Further, it has been found by North that egg yolk added to milk greatly increases the electric charge on the fat globules. The absorption of the lecithin-protein complex on the fat globules in an ice-cream mix may be expected to increase the negative potential on the globules. The higher charge on the globule will prevent fat clumping to a greater extent. Both the better adhesion of the serum to the fat globules and the decreased clumping are conducive to a higher whipping ability.

Although the data are not shown in the tables, there was invariably less fat clumping in the egg-yolk mixes than in the mixes without egg yolk.

EGG AND SOYBEAN LECITHINS

Studies were made of the effect of egg lecithin and of soybean lecithin on the whipping properties of mixes made from butter, dried skim milk, and water. To these mixes various amounts up to 0.2 percent of egg and soybean lecithin were added. The lecithin was incorporated into the mix by thoroughly mixing it with about a pound of melted butter. All of the mixes were viscolized with a two-stage valve at 2,000 pounds pressure at the pasteurization temperature. The results are shown in table 5.

It will be noted in table 5 that the addition of egg lecithin up to 0.2 percent exerted a beneficial effect on the whipping properties of the ice-cream mix made with butter, dried skim milk, and water. A saving of 4.83 minutes in the time required to secure 100 percent overrun was obtained when 0.17 percent of egg lecithin was added. On the other hand, soybean lecithin in amounts ranging from 0.1 to 0.2 percent had a detrimental effect on the whipping properties and the percentage of overrun obtained. The larger the percentage of soybean lecithin used, the more noticeable was this effect.

The flavor of the ice cream was lowered with each increment increase of commercially prepared egg or soybean lecithin.

TABLE 5.—Effect of varying amounts of egg lecithin and soybean lecithin on the whipping properties of the mix and quality of the ice cream

EGG LECITHIN

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3-5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
	<i>Minutes</i>				
Butter; dried skim milk; water.....	11 43	42.50	23.33	5	70.83
Butter; dried skim milk; water; 0.01 percent egg lecithin.....	9 30	42.50	23.33	5	70.83
Butter; dried skim milk; water; 0.05 percent egg lecithin.....	9 00	41.47	23.33	5	69.80
Butter; dried skim milk; water; 0.10 percent egg lecithin.....	8 36	36.00	23.33	5	64.33
Butter; dried skim milk; water; 0.15 percent egg lecithin.....	7 55	30.33	23.33	5	58.66
Butter; dried skim milk; water; 0.17 percent egg lecithin.....	6 60	20.66	23.33	5	48.99
Butter; dried skim milk; water; 0.20 percent egg lecithin.....	8.55	19.66	23.33	5	47.99

SOYBEAN LECITHIN

Butter, dry skim milk; water.....	9 50	42 16	23 16	5	70 32
Butter, dried skim milk; water; 0.1 percent soybean lecithin ^a	12 00	41 33	23 83	5	70 16
Butter; dried skim milk; water; 0.15 percent soybean lecithin ^a	(5)	41 09	22 50	5	68 50
Butter; dried skim milk; water; 0.17 percent soybean lecithin ^a	(5)	40 33	22 16	5	67 49
Butter, dried skim milk; water; 0.2 percent soybean lecithin ^a	13 00	(4)	21 50	5	65.50
	12 00	39 00			

^a Amounts of lecithin used were calculated on the basis of the soybean lecithin being 55 percent pure according to the method used for determining the purity of egg lecithin.

^b Maximum percentage of overrun obtainable, 61.

^c Maximum percentage of overrun obtainable, 57.

^d Maximum percentage of overrun obtainable, 54.

Why the results obtained with the two types of lecithin were so different is not known. The fact that the fatty acids found in soybean lecithin are not all the same as those in egg lecithin (8) may have influenced the results to some extent. Moreover, commercial soybean lecithin differs from egg lecithin in that it is a mixture of vegetable lecithin and a neutral oil, the oil being added chiefly to increase the solubility and stability of the lecithin in those products in which it is used. Vegetable lecithin is understood to have associated with it a carbohydrate group ³ and this might conceivably influence its colloidal behavior. Richardson (20) and Osborne and Campbell (13) consider that egg lecithin is present both free and in combination with the egg-yolk proteins. Sonner (21, p. 308-09) states:

It suggests the hypothesis that the egg lecithin, because of its fatty nature (glycerol and two fatty acid radicals in the molecule), will dissolve in the fat globules but will remain oriented at the surface because of the other groups in the lecithin molecule having polar properties (affinity for water). If the egg lecithin is in combination with egg proteins, then this would result in the formation of an adsorption film around the fat globules consisting of the lecithin-protein complex in addition to the casein and other substances usually adsorbed in ordinary mixes. This should leave the surface of the fat globules in such a condition that the serum will adhere more tenaciously and the mix should therefore whip better * * *. The fat globules represent a less serious point of weakness in the lamellae and therefore better whipping results.

³ EICHBERG, J. Letter to senior author, Mar. 26, 1932.

Sommer's hypothesis seems logical, for the affinity of water being greater for proteins than for fat, the serum which composes the lamellae would adhere more readily to the fat globules if the outer layer of the oriented lecithin molecules was composed of protein.

Nitrogen determinations were made on the egg lecithin used in these experiments and an average of 0.079 percent of nitrogen was found in excess of that theoretically present in the accepted formula for lecithin. By using the factor 6.25 (6), this quantity of excess nitrogen is found to be equivalent to 0.5 percent of protein which could be present in combination with the lecithin as an adsorption film around the fat globule and improve the whipping properties, according to Sommer's hypothesis.

BUTTERMILK

Caulfield and Martin (4) attribute the failure of mixes containing butter to whip as readily as those containing cream to the churning process and not to the treatment the butter mixes receive. They found that the use of buttermilk in the mix resulted in only a slight improvement in the whipping properties. Tracy and Ruehe (22) also found that the longer time required for whipping in the case of butter mixes was not due to the absence of the buttermilk.

Whitaker (24) has obtained data which show that the addition of buttermilk to the butter mix does materially benefit the whipping properties and he suggests that the beneficial effect of the buttermilk is due to the lecithin that it contains. Martin (9) also has suggested that the lack of lecithin in butter mixes may have a bearing on their inferior whipping qualities. He states that the addition of powdered sweet-cream buttermilk has been found to be beneficial to the whipping properties.

The foregoing findings as to the effect of buttermilk in the butter mix, and the pronouncedly beneficial effect of egg lecithin noted in the present experiments, suggested the desirability of a further study of the effect of buttermilk on the whipping properties of butter mixes. Accordingly, fresh 40 percent cream was churned according to accepted methods. Some of the original cream, unsalted butter, and the buttermilk were used with dried skim milk and water to make ice-cream mixes. The results obtained are presented in table 6.

TABLE 6.—*Effect of buttermilk on the whipping properties of the mix and quality of the ice cream*

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3-5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
Cream; dried skim milk; water.....	Minutes 8.69	43.58	23.90	5	72.48
Butter; dried skim milk; water.....	12.00	43.26	23.90	5	72.10
Butter; dried skim milk; water; buttermilk * from above-mentioned cream.....	10.00	43.59	23.90	5	72.49

* Buttermilk supplied 38.5 percent of serum solids in 2 experiments and 1.6 percent in another experiment. The latter amount just equals the amount of serum solids supplied when cream is used as the source of fat.

Table 6 shows that the return of the buttermilk to the butter mix improved its whipping qualities. An average saving of 2 minutes to obtain the desired yield was effected by the addition of the buttermilk. The total score of the ice cream was slightly higher than that of the control mixes.

There has been considerable evidence that the phospholipids in milk are in combination with plasma protein (7, 14, 15, 16, 17), and Palmer and Wiese (17) have recently shown this to be true. Skim milk and butter contain only a very small amount of lecithin as compared with that contained in buttermilk (1). In view of the fact, then, that the lecithin is in combination with plasma proteins and that buttermilk contains the preponderance of the lecithin originally in the cream, and in view of the specific results obtained with egg lecithin and buttermilk in these experiments, the failure of butter mixes to whip as readily as cream mixes may be explained as due to a lack of the lecithin-protein complex. Sommer's (21) theory of the whipping phenomenon and his hypothesis regarding the lecithin-protein adsorption film around the fat globules are necessary adjuncts to this explanation.

SUMMARY AND CONCLUSIONS

The effects of various egg products, soybean lecithin, and buttermilk upon the whipping properties of ice-cream mixes and the quality of ice cream made from unsalted butter, dried skim milk, and water are presented.

The addition of 0.5 percent of dried egg yolk to butter-dried skim milk mixes greatly improved their whipping properties. The beneficial effect of egg yolk was more pronounced in mixes made with butter and dried skim milk than in mixes made with cream and dried skim milk.

The whipping properties of butter-dried skim milk mixes were improved equally by Chinese or domestic dried egg yolk. Dried whole egg improved whipping but did not give as good results as dried egg yolk. Sweetened frozen egg yolk materially improved the whipping properties of the mix but not to the same extent as dried egg yolk.

Of the various fractions separated from dried egg yolk, the vitellin was detrimental to the whipping properties. The egg-yolk salts exerted a slightly beneficial effect, as did also the portion of the yolk containing the salts and the proteins. The most pronounced beneficial effect was obtained with the portion of the yolk containing the lecithin. It is believed that certain of the egg-yolk proteins are in combination with the lecithin.

The addition of certain percentages of egg lecithin to butter-dried skim milk mixes gave decidedly beneficial results. Soybean lecithin, on the other hand, was detrimental to the whipping properties of the mix.

Sweet-cream buttermilk added to the butter-dried skim milk mix improved the whipping properties.

It is believed that the beneficial results of egg yolk on the whipping properties of butter mixes are due to a lecithin-protein complex in the yolk and that a similar lecithin-protein complex in buttermilk is responsible for its beneficial effect.

The data seem to indicate that the failure of butter mixes to whip as readily as cream mixes is due to the absence of the buttermilk and its lecithin-protein complex.

When dried skim milk and butter are used in making commercial ice-cream mixes, it is recommended that 0.5 percent of good quality dried egg yolk be used to improve the inferior whipping properties of such mixes.

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EFFECT OF METHOD OF SOWING ON THE YIELD AND ROOT AND TOP DEVELOPMENT OF SWEETCLOVER IN THE RED RIVER VALLEY ¹

By R. S. DUNHAM ²

Agronomist, Northwest Experiment Station, University of Minnesota

INTRODUCTION

Sweetclover is a particularly important crop in the Red River Valley. It is especially adapted to the calcareous soils common to this area, it fits well into the average farm program and serves a variety of uses. It is commonly grown for pasture and seed production and less frequently for hay, but in connection with these uses it is generally believed to have value from the standpoint of soil improvement. In fact, its use primarily as a soil-improvement crop is increasing.

Extensive studies of the life history of sweetclover have been made in Ohio under humid conditions and a long growing season, and in Saskatchewan, a region of limited moisture supply and a short growing season. Climatic conditions in northwestern Minnesota are somewhat intermediate between these two.

It appeared desirable, therefore, to obtain definite information about the behavior of this crop in the Red River Valley, and a study was therefore made at the Northwest Experiment Station near Crookston, Minn. This paper presents data on yields of roots and tops harvested at various stages of growth as found in two types and two varieties of sweetclover sown with and without companion crops. It also presents some data on the nitrogen content of tops and roots.

REVIEW OF LITERATURE

It is generally agreed that where moisture is a limiting factor, the best stands of sweetclover are obtained when sown alone. However, except in very dry regions and on some light soils, seeding with companion crops is a general farm practice. As a result of investigations on the root development of sweetclover in Saskatchewan, Canada, where the precipitation during the growing seasons of 1928 and 1929 was 10.04 and 7.28 inches, respectively, Kirk ³ concluded:

One could improve the soil as much in one season by growing sweetclover without a nurse crop as in 8 or 10 years by growing it with grain.

The competition of plants for survival and its influence on root development and top growth has been the subject of study by a few

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² The author is indebted to Dr. H. K. Hayes, chief of the Division of Agronomy and Plant Genetics, and to A. C. Army, associate agronomist, under whose direction the investigational work was planned; to Dr. C. H. Bailey, Division of Agricultural Biochemistry, under whose direction the nitrogen determinations were made; to J. C. Hyde, Division of Soils, Minnesota Agricultural Experiment Station, for technical description of the soil on which the plots were laid out; and to Dr. F. R. Immer, Division of Sugar Plant Investigations, U. S. Department of Agriculture, for his assistance with the mathematical phases of the problem.

³ KIRK, L. E. SWEET CLOVER AND SOIL IMPROVEMENT. Saskatchewan Univ. Col. Agr. Leaflet. 1931. [Mimeographed.]

investigators. Willard (14)⁴ found that regardless of the initial stand, the final stand in mature sweetclover was about 30 plants per square yard. Piper (10) gives 7 plants per square foot or 63 per square yard as a perfect stand of mature sweetclover. McCool (8) in Michigan found striking differences between the root development of sweetclover plants thinned and unthinned.

Although the characteristics of the top growth and hay yields of the common sweetclover types and varieties are generally known, fewer investigations of the root growth and chemical composition have been made. Willard (14) found that biennial yellow produced a larger proportion and usually more roots than did the white sweetclover in the fall of the first year. Grundy County produced more roots and less tops than did the common white in the fall of the first year and less total yield the second year. Kirk compared several sweetclover varieties including white- and yellow-blossom types and found that they did not show any marked difference in the fertilizing value of their root material. Both Kirk (7)⁵ and Willard (14) found there were no important differences in the composition of the tops of white and yellow sweetclover at corresponding stages. Arny and McGinnis (2) reported trials made in four sections of Minnesota. In all cases biennial white sweetclover outyielded biennial yellow in tops and roots in October of the first year. As an average of the 4 trials, the percentage of protein in the tops and the roots was approximately the same for the 2 types.

Hopkins in Illinois early recognized the promise of sweetclover as a green manure crop. He introduced it into the rotations on most of the 40 experimental fields throughout the State and selected it as the crop upon which to base his foundation work for restoring the soils of Greece. Bauer and De Turk, as reported by Keilholz (6), found from greenhouse studies with soil low in organic matter that—

when sweet clover was grown on such soil the crop-producing power of it was improved, even tho both tops and roots were removed. The mere growing of the sweet clover crop helped the succeeding crop. The greatest benefit was secured, however, where the sweet clover was returned as a green manure.

Kirk⁵ wondered—

why sweet clover plowed down can increase the yields of corn 50 per cent in the corn belt, while its beneficial effects on the succeeding crop of grain can rarely be seen in the prairie plains sections of Western Canada.

The root development of plants has been found by various investigators (9, 11, 12, 13) to depend on the type of soil, the water content of the soil, the depth of the water table, the species of plant, and the amount of top growth of the plant.

Aside from the development of roots as affected by various factors of environment, the study of root reserves also has received attention. Willard (14, 15) and Kirk⁵ in separate investigations have described the behavior of root reserves in sweetclover. Both investigators report a rapid decline in nitrogen and total dry matter as soon as growth begins in the spring of the second year.

The nitrogen content of types and varieties of sweetclover at various stages of growth and on different soils is of importance in a study of this plant as a green manure. Kirk (7, p. 391) found that the average

⁴ Reference is made by number (italic) to Literature Cited, p. 994.

⁵ Kirk, L. E. See footnote 3.

protein content does not differ significantly for the different sweetclover types, and Williams in Ohio (16, p. 21) reported that there were no important differences in the composition of white and yellow sweetclover at corresponding stages. Arny and McGinnis (2) found a maximum difference between biennial white and biennial yellow of 0.30 percent of nitrogen in the tops and 0.32 percent in the roots. Although the development of both tops and roots is affected materially by the type of soil (2, 4, 8), it seems possible that the total amount of nitrogen in an acre of sweetclover on different soils varies more because of a difference in yield than a difference in composition.

In comparisons of sweetclover at various stages of growth, Willard (14) found that the nitrogen content of the tops and roots in the fall of the first year did not vary greatly from 3 percent. In the early spring of the second year the roots contained from 4 to 4½ percent and in July about 1 to 1½ percent. Jones and Garrard (5) showed a decrease from 3.2 to 2.7 in the percentage of nitrogen in roots sampled April 26 and July 10, respectively. Kirk (7) found that the values for protein (in the tops) were almost identical in a fall cutting the first year, a first hay crop the second year, and a second hay crop the second year as an average of five "types" of sweetclover.

MATERIALS AND EXPERIMENTAL PROCEDURE

The surface soil of the plots used in this investigation is a very fine sandy loam, usually black to a depth of about 8 inches. The subsurface soil is a very fine sand and the subsoil a very calcareous lacustral clay. The depth to the clay varies from 12 to 40 inches. Hydrochloric acid will usually produce effervescence at the surface and always in the clay.

The field on which the plots were laid out had been cropped previously as follows: 1926, field peas; 1927, winter rye; 1928, barley; 1929, fallow; and 1930, potatoes.

Precipitation and temperature data are shown in table 1. The total precipitation for 1931 and until July 14, 1932, was less than average which under the drainage conditions of the station farm favored the experimental work. The mean temperatures were higher than the average.

TABLE 1.—*Precipitation and mean temperature at Crookston, Minn., during 1931–32 and 10-year average, 1920–29*

Item	Jan.	Feb	Mar	Apr	May	June	July	Aug	Sept.	Oct	Nov	Dec	Total
Precipitation:	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>
1931	0.06	0.09	0.70	0.05	3.09	2.64	3.06	1.07	2.33	2.59	1.51	0.10	17.29
1932	.22	.54	.36	1.46	2.23	1.80	*1.01						
10-year average (1920–29)	.31	.52	.37	1.61	2.91	3.55	2.41	1.89	2.09	1.26	1.00	.36	18.28
Mean temperatures:	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>
1931	17.0	25.6	27.4	44.6	53.2	67.8	70.9	67.6	63.5	49.5	32.4	19.1	44.9
1932	11.6	10.7	18.0	43.4	57.2	69.2	*71.6						
10-year average (1920–29)	5.4	10.6	24.5	41.5	55.0	62.9	68.8	66.7	58.0	44.9	26.2	10.7	39.6

* Until July 14 when last harvest of roots was made.

Two species or types of sweetclover, biennial white (*Melilotus alba*) and biennial yellow (*Melilotus officinalis*), and a variety of each, Grundy County and Albotrea, were used.

Biennial white is most commonly grown in the valley. It is a coarse rank-growing type used for the production of both seed and forage and for soil improvement.

Biennial yellow is finer stemmed and earlier than biennial white. Usually it yields less in hay. It is less desirable for pasture because of its early blossoming and its habit of producing seed close to the ground which shatters and reseeds the crop. It is less widely grown than the white but occurs throughout the valley largely because of mixture in seed of the common white.

Grundy County is a biennial dwarf white-blossom variety developed in Grundy County, Ill. It was at one time quite extensively used as a seed crop in the valley, but more recently it has largely lost favor among the farmers because of (1) a limited demand for the seed in this section, (2) its smaller yield of forage, and (3) the prevalent idea that it does not have so extensive a root system as either the biennial white or biennial yellow and therefore is of less value for soil improvement.

Albotrea is a yellow-blossomed biennial and probably a variety of the common yellow (*Melilotus officinalis*). The original plant was found growing along the roadside in Canada. At present Albotrea is of no commercial importance in the Red River Valley.

Each of the four kinds of sweetclover was sown in five ways (table 2): (1) With wheat (*Ceres*) as a companion crop, (2) with oats (*Anthon*) as a companion crop, (3) with barley (*Trebi*) as a companion crop, (4) without companion crop sown early (Apr. 29, 1931), and (5) without companion crop sown late (June 10, 1931). The companion crops were all planted April 29, 1931, at the usual rates of seeding; the sweetclover seed was sown at the rate of 12 pounds per acre.

Yields of both roots and tops were calculated on the basis of square-yard samples dried moisture free. Six square yards were taken from each plot for yields of tops and three for roots and stubble. The square-yard measures used are described by Arny and Steinmetz (3).

TABLE 2.—Sweetclover plots and companion crops sown

Sweetclover	Series 1					Series 2				
	Wheat	Oats	Barley	Alone early	Alone late	Wheat	Oats	Barley	Alone early	Alone late
Albotrea.....	1	2	3	4	5	6	7	8	9	10
Biennial yellow.....	1a	2a	3a	4a	5a	6a	7a	8a	9a	10a
Grundy County.....	1b	2b	3b	4b	5b	6b	7b	8b	9b	10b
Biennial white.....	1c	2c	3c	4c	5c	6c	7c	8c	9c	10c

In the first digging of roots made at the harvest of companion crops, the square yards were distributed over the plots at random. It was apparent, however, in these studies that this was not the best method of locating the areas to be dug. Since the stand of sweetclover was variable on the plots, it seemed best to group the square yards close together so that comparisons could be made between adjacent square-yard areas. Three areas of uniform stand on each

plot were selected and the square yards of the four subsequent diggings taken from these areas. The 3 square yards from each plot were bulked and treated as one sample. Figure 1 illustrates the arrangement and approximate location of the areas on the plots.

Harvests of tops and roots were made (1) at the time the companion crops were removed, (2) in October of the first year, (3) in May of the second year, (4) at the time of cutting the first hay crop, and (5) at the time of cutting the second hay crop.

In the first digging all roots were found in the upper 7 inches of the soil so that a 1-foot slice was amply deep. When the vertical roots tapered to a millimeter or less in diameter, no attempt was made to reach their extremities. Obviously, the yields of the second-year diggings are somewhat lower than they should be, but the findings of Willard (14), Army (2), and Kirk⁶ show this loss in weight to be small.

In addition to yields of tops and roots, the nitrogen content of the roots of Albotrea and biennial white and the tops of biennial white only was determined. In view of the fact that several investigators (2, 15)⁶ have found no important difference in composition between sweetclover types, it was deemed inadvisable to extend the number of nitrogen determinations in this study.

In digging the roots it was impossible to recover all root nodules with the facilities available. To make the samples comparable, therefore, all nodules were stripped from the roots. Unquestionably this loss introduces an error in the nitrogen content of the roots, but it was unavoidable under the circumstances.

EXPERIMENTAL RESULTS

Yields of tops, roots, stubble, and the entire plant are reported in table 3. For ease of comparison, the table is discussed under these separate parts. Biometrical analyses of the data were made by Student's method (1) since any 2 adjacent square yards may be considered as paired comparisons. In the discussion of these comparisons, odds of 20 to 1 have been chosen arbitrarily as the minimum for indicating significant differences.

YIELDS OF TOPS

Wheat appears here to be better than oats or barley as a companion crop for each kind of sweetclover. Furthermore, no important difference in behavior is noticeable between the types and varieties when sown with either oats or barley. No harvests of sweetclover sown alone were made at the time of cutting the companion crops on the other plots nor was this sweetclover cut. Therefore the October yields of tops from the plots sown alone should not be compared with those from the sowings with companion crops, since the former had been allowed to grow all season whereas the latter had been cut with an 8-inch stubble in August.

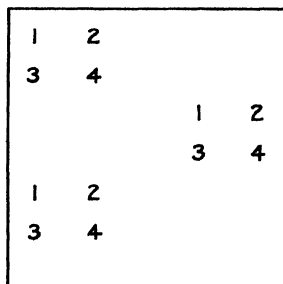


FIGURE 1.—Arrangement and location of square-yard areas on the plots. The times of harvest indicated are 1, October 1931; 2, May 1932, 3, at time of cutting the first hay crop, June 10 to 14, 1932; 4, at time of cutting the second hay crop, July 14 to 19, 1932.

⁶ Kirk, L. E. See footnote 3.

TABLE 3.—Average yield per acre of tops, roots, and stubble, and total dry weight (pounds) of 4 kinds of sweetclover when sown alone early and alone late, and with companion crops

Method of sowing	Time of harvest	Alborea			Biennial yellow			Grundy County			Biennial white		Average total weight, 4 kinds
		Tops	Roots	Stubble	Total	Tops	Roots	Stubble	Total	Tops	Roots	Stubble	Total
Alone in April	October	1,771	3,540	409	5,720	1,823	2,941	338	5,102	1,007	2,124	304	3,445
	May	1,327	1,850	(*)	3,177	1,176	1,697	(*)	2,873	1,076	1,184	(*)	2,260
	First hay crop	1,057	1,829	1,430	4,346	1,235	1,573	1,530	4,378	1,294	1,392	1,636	4,322
Alone in June	(Second hay crop)	1,564	1,437	820	3,821	1,656	1,753	978	4,387	1,746	1,261	1,012	4,019
	October	957	2,012	207	3,176	858	1,927	192	2,987	375	863	86	1,324
	May	821	1,132	(*)	1,973	731	1,065	(*)	1,846	560	582	(*)	1,142
With wheat	First hay crop	797	1,103	1,117	3,017	904	913	1,195	3,012	1,116	913	1,210	2,039
	(Second hay crop)	1,717	932	569	3,238	1,735	714	661	3,130	1,866	563	720	3,179
	(At grain harvest)	804	508	508	1,830	536	1,222	289	927	204	51	138	383
With oats	October, first year	306	1,742	247	2,295	265	1,531	233	2,023	183	1,081	191	1,455
	May, second year	1,097	1,065	(*)	2,162	1,060	997	(*)	2,077	763	640	(*)	1,403
	First hay crop, second year	1,334	1,027	1,254	3,615	1,540	1,080	1,291	3,921	1,694	857	1,246	3,777
With barley	(Second hay crop)	1,562	870	818	3,250	1,711	980	775	3,526	1,927	703	966	3,596
	(At grain harvest)	330	186	186	601	160	45	157	362	81	33	136	260
	October	212	985	180	1,377	177	1,010	136	1,323	88	701	147	1,936
With oats	May	615	646	(*)	1,261	669	626	(*)	1,325	471	508	(*)	1,079
	First hay crop	829	829	901	2,507	1,132	771	1,040	2,943	944	478	637	2,359
	(Second hay crop)	1,443	565	565	2,573	1,367	628	628	2,843	1,707	455	739	2,921
With barley	(At grain harvest)	319	172	286	777	209	70	218	497	105	42	141	288
	October	252	1,392	219	1,963	219	1,107	138	1,454	163	549	134	1,108
	May	704	837	(*)	1,541	732	691	1,423	2,839	994	594	(*)	2,000
With barley	First hay crop	752	861	978	2,591	1,021	731	1,067	2,839	894	563	1,069	2,566
	(Second hay crop)	1,527	725	537	2,789	1,704	724	702	3,130	1,576	601	793	2,970

* Stubble not separated; included in weight of tops.

If the October yields are disregarded for these reasons, sowing with wheat likewise appears better than sowing alone in June for all types and varieties at all harvests except possibly in the case of the second hay crop of Albotrea. A comparison of the sowings with wheat and those sown alone in April reveals some variations between the sweetclovers. Biennial white yielded more in all 3 harvests from the sowing with wheat than from the plots sown alone, Grundy County and biennial yellow yielded more in 2 harvests, and Albotrea in only 1. An interesting observation is the fact that Albotrea, biennial yellow, and biennial white sown alone in April produced more top growth by October of the first year than in any later harvest.

No one kind of sweetclover yielded significantly better than any other at all harvests. At grain harvest, in October of the first year, and in May of the second year, Albotrea, biennial yellow, and biennial white had made larger top growths than Grundy County, although the odds that biennial white was superior in May are too low to be significant. As between Albotrea, biennial white, and biennial yellow at these harvests, there was no consistent superiority of one over the others. In the first hay crop both biennial yellow and Grundy County yielded more than Albotrea. Biennial white outyielded Albotrea and biennial yellow in both hay crops and also outyielded Grundy County in the second hay crop.

YIELDS OF ROOTS

The yields of roots given in table 3 are graphically illustrated in figure 2. Harvests from the plots sown alone were not made, so comparisons of sowing with and without a companion crop at this period are not possible.

In table 3 the yields of roots with wheat as the companion crop are larger for each kind of sweetclover at each harvest except one than with oats or barley. There appears to be no important difference between the sweetclovers when sown with barley or oats. In only 2 of the 32 harvests were the yields of roots larger from the sweetclover sown with oats than from that sown with barley. In all harvests of each kind of sweetclover, the yields of roots were larger from the sweetclovers sown alone in April than from the sowing with wheat. A comparison of the sweetclover sown alone in June with that sown with wheat shows no significant difference in yield of roots.

In a comparison of types and varieties in yields of roots, it is at once apparent that Grundy County made a root development inferior to the other kinds. Only at the time of the second hay crop, the odds that Albotrea yielded more are not significant. Both of the yellow-blossom types made a larger root development the first year than the biennial white. In the second year there is no significant difference between them. No one type or variety was superior to the other three throughout both seasons.

TOTAL WEIGHT OF PLANT

In the last column of yields in table 3 the combined weight of tops, roots, and stubble are reported as total weight. The weights of stubble are relatively high in the harvests at time of cutting the grain crop and at the first cutting of hay. At both times an 8-inch stubble was left because of cutting with a grain binder in the first instance and to avoid killing plants by too close cutting in the second case. The second crop of hay was cut with a 3-inch stubble.

On all plots where a companion crop was used and likewise where sown alone in June, the maximum total dry weight was produced generally at the time of cutting the second hay crop, but in 4 instances out of 16 at the time of cutting the first crop. Where sown alone in April, the maximum weight occurred in October of the first year among

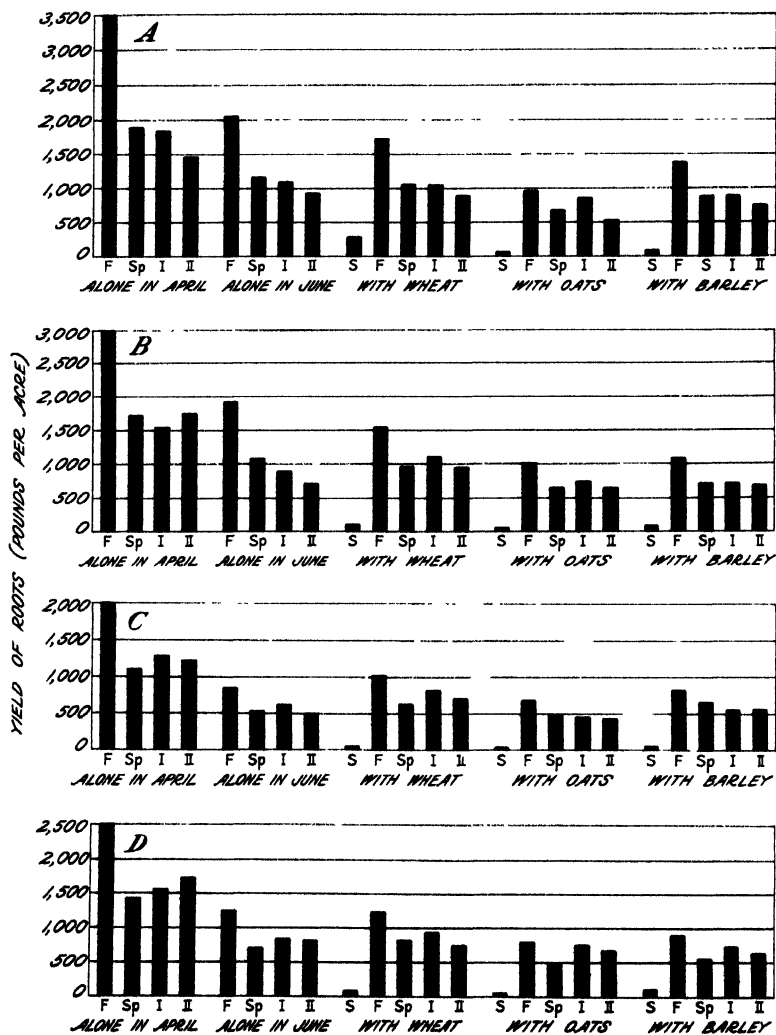


FIGURE 2.—Yields of Albortres (A), biennial yellow (B), Grundy County (C), and biennial white (D), sweetclover roots at various periods, and when sown with and without companion crops: S—at grain harvest; F=October, first year; Sp=May, second year; I=first hay crop; II=second hay crop.

all types and varieties except Grundy County. From the standpoint of the entire plant, i.e., roots, stubble, and tops, the odds are not always significant that wheat was a better companion crop than oats or barley. However, the trend of the comparisons indicates this to be the case, since in no instance was sowing with either oats or barley superior to sowing with wheat. That sowing alone in April resulted

in larger total dry weights of the crop than sowing with wheat is definitely indicated. Sowing alone in June resulted in decidedly lower yields than sowing alone in April but was not significantly better or worse than sowing with wheat.

The data in table 3 show that the total dry weight of the first year was larger for the 2 yellow-blossom sweetclovers than for the 2 white. Subjected to Student's method (1) of comparison, the yellow-blossom sweetclovers yielded significantly more than the white except in the comparison of biennial white with biennial yellow. In only two comparisons were the odds significant throughout both seasons. Alborea yielded significantly better than biennial white in the first 3 harvests and definitely less in the 2 hay crops. Biennial white yielded significantly greater during both seasons than Grundy County.

STAND IN RELATION TO METHODS OF SOWING

A determination of the stand of sweetclover following the five methods of sowing was made by counting the plants on 3 square yards of each plot and a like number on a duplicate plot. In table 4, comparisons of methods of sowing are shown with average differences and odds that the differences are significant. The comparisons are made using the four types and varieties of sweetclover. In a comparison of the desirability of the companion crops, only 3 counts out of 15 are significantly different. The number of plants in May, the second year, and at the time of the second hay crop when sown with wheat was greater than that sown with oats. The count of the sweetclover plants sown with barley was greater than where sown with oats at the time of the second hay crop. Sweetclover sown with wheat resulted in a larger stand in May of the second year than when sown alone in April and also larger than when sown alone in June.

TABLE 4.—Comparison of the stands of 4 kinds of sweetclover when sown alone early and alone late and with companion crops

Methods of sowing	Time of harvest	Average difference	Approximate odds
		<i>Number of plants per square yard</i>	
Barley v. oats.	At grain harvest.....	2.8	—1.5:1
	October, first year.....	5.8	1.8:1
	May, second year.....	4.8	—1.5:1
	First hay crop, second year.....	—7.5	2.3:1
	Second hay crop, second year.....	42.2	47.8:1
Wheat v. sweetclover alone in April.	October, first year.....	27.8	6.2:1
	May, second year.....	66.0	70.4:1
	First hay crop, second year.....	16.5	4.1:1
	Second hay crop, second year.....	10.3	2.3:1
	At grain harvest.....	21.0	4.1:1
Wheat v. barley.	October, first year.....	21.8	4.1:1
	May, second year.....	21.4	2.8:1
	First hay crop, second year.....	7.1	1.8:1
	Second hay crop, second year.....	—6.5	1.8:1
	At grain harvest.....	23.6	6.2:1
Wheat v. oats.	October, first year.....	27.5	7.8:1
	May, second year.....	69.1	59.2:1
	First hay crop, second year.....	—0.38	—1.5:1
	Second hay crop, second year.....	35.7	1,990:1
	October, first year.....	23.8	5.2:1
Wheat v. sweetclover alone in June.	May, second year.....	45.8	26.8:1
	First hay crop, second year.....	19.1	7.8:1
	Second hay crop, second year.....	31.2	9.4:1

Since the yields of both roots and tops reported previously showed wheat to be definitely a better companion crop than oats or barley, it is apparent that yield was not dependent upon the number of plants per measured area in the first year.

The variation in stand at various periods and under different methods of sowing is shown in table 5. In all cases when sown with a companion crop, the stand increased from the time of grain harvest to October of the first year. With only two exceptions, the stand decreased in number of plants from October of the first year to July of the second year, at which time the second crop of hay was cut. The maximum decrease in stand from October or May till July was 51.48 percent, occurring when biennial white was sown with wheat. The average decrease in stand for all types and varieties was: With wheat, 34.77 percent; with oats, 36.61 percent; with barley, 25.49 percent; sown alone in April, 22.71 percent; and sown alone in June, 35.45 percent.

TABLE 5.—Average number of sweetclover plants per square yard at successive periods for 4 kinds of sweetclover sown alone early and alone late and with companion crops

Method of sowing and kind of sweetclover	At grain harvest	October, first year	May, second year	First hay crop	Second hay crop
With wheat					
Alborea	183	265	262	174	195
Biennial yellow	101	200	302	202	186
Grundy County	74	227	163	188	133
Biennial white	156	186	237	142	115
Average	128.5	219.5	241.0	176.5	157.2
With oats					
Alborea	107	255	201	235	146
Biennial yellow	92	200	191	185	147
Grundy County	118	150	163	147	94
Biennial white	107	180	136	141	98
Average	106.0	191.2	172.7	177.0	121.2
With barley					
Alborea	128	283	284	216	153
Biennial yellow	134	207	236	223	205
Grundy County	71	173	211	130	191
Biennial white	102	129	148	109	106
Average	108.7	198.0	219.7	169.5	163.7
Alone in April:					
Alborea		164	187	172	161
Biennial yellow		235	159	147	128
Grundy County		170	179	160	173
Biennial white		192	176	162	126
Average		190.2	175.2	160.2	147.0
Alone in June:					
Alborea		210	190	207	126
Biennial yellow		239	251	167	123
Grundy County		173	185	125	127
Biennial white		159	155	131	128
Average		195.2	195.2	157.5	126.0
Average of 4 kinds		198.8	200.8	168.2	143.0

ROOT DEVELOPMENT IN RELATION TO LIFE HISTORY OF THE PLANT

The dry weights of the roots per acre at various stages in the life history of the sweetclover plant and under different methods of sowing are reported in table 3 and graphically illustrated in figure 2. Of striking significance is the extremely small weight of the roots dug following the removal of the companion crops as reported in table 3 and illustrated in figure 2. Without a single exception among the four types and varieties or under any method of sowing, the maximum weight of roots was produced by October of the first year. The harvests in May were taken when the sweetclovers had made from 4 to 6 inches top growth. In every case the weight of roots was less than in the

preceding fall. Generally there was some increase in the weight of roots from May till the time of cutting the first hay crop, and in no case was there a large decrease. In only one half of the trials did the weights of roots from the last digging represent the minimum for all harvests subsequent to that at grain harvest.

TABLE 6.—Average weight (grams) at successive periods of roots from single plants of 4 kinds of sweetclover sown alone early and alone late, and with companion crops

Method of sowing and time of harvest	Alborea			Binennial yellow			Grundy County			Biennial white			Average of 4 kinds
	Plot 4	Plot 9	Average	Plot 4a	Plot 9a	Average	Plot 4b	Plot 9b	Average	Plot 4c	Plot 9c	Average	
Sown alone in April:													
October, first year	1 91	2 15	2 03	1 12	1 21	1 16	1 23	1 14	1 18	1 28	1 14	1 21	1 40
May, second year	1 29	.73	1 01	1 02	.98	1 00	.66	.59	.62	.81	.74	.78	.85
First crop, second year	.85	1 33	1 00	1 13	.93	1 03	.65	.96	.80	.75	1 06	.90	.96
Second crop, second year	.82	.84	.83	1 29	1 26	1 28	.97	.53	.75	1 37	1 24	1 30	1 04
	Plot 5	Plot 10	Average	Plot 5a	Plot 10a	Average	Plot 5b	Plot 10b	Average	Plot 5c	Plot 10c	Average	
Sown alone in June:													
October, first year	0 63	1 14	0 88	0 73	0 77	0 75	0 31	0 60	0 46	0 41	1 11	0 76	0 71
May, second year	.38	.76	.57	.44	.36	.40	.19	.38	.28	.35	.54	.44	.42
First crop, second year	.40	.60	.50	.49	.52	.50	.42	.50	.46	.49	.70	.60	.52
Second crop, second year	.53	.86	.70	.63	.47	.55	.40	.43	.42	.54	.69	.62	.57
	Plot 1	Plot 6	Average	Plot 1a	Plot 6a	Average	Plot 1b	Plot 6b	Average	Plot 1c	Plot 6c	Average	
Sown with wheat:													
Grain harvest	0 31	0 10	0 20	0 30	0 08	0 19	0 027	0 068	0 048	0 052	0 054	0 053	0 12
October, first year	.60	.65	.62	.85	.59	.72	.49	.42	.46	.82	.48	.65	.61
May, second year	.44	.32	.38	.37	.25	.31	.45	.32	.38	.46	.24	.35	.36
First crop, second year	.56	.55	.56	.64	.40	.52	.43	.41	.42	.76	.54	.65	.54
Second crop, second year	.39	.46	.42	.85	.28	.56	.77	.36	.56	.72	.58	.65	.55
	Plot 2	Plot 7	Average	Plot 2a	Plot 7a	Average	Plot 2b	Plot 7b	Average	Plot 2c	Plot 7c	Average	
Sown with oats:													
Grain harvest	0 13	0 048	0 089	0 071	0 030	0 050	0 027	0 026	0 026	0 024	0 031	0 028	0 05
October, first year	.34	.38	.36	.62	.36	.49	.50	.38	.44	.42	.52	.47	.44
May, second year	.33	.28	.30	.50	.23	.36	.34	.24	.29	.37	.34	.36	.33
First crop, second year	.35	.30	.32	.68	.24	.46	.34	.28	.31	.65	.42	.54	.41
Second crop, second year	.31	.48	.40	.54	.32	.43	.63	.35	.49	.81	.63	.72	.51
	Plot 3	Plot 8	Average	Plot 3a	Plot 8a	Average	Plot 3b	Plot 8b	Average	Plot 3c	Plot 8c	Average	
Sown with barley:													
Grain harvest	0 16	0 10	0 13	0 081	0 038	0 060	0 063	0 048	0 056	0 080	0 036	0 058	0 08
October, first year	.47	.46	.46	.56	.46	.51	.40	.54	.47	.66	.62	.64	.52
May, second year	.30	.27	.28	.31	.24	.28	.28	.32	.30	.35	.41	.38	.31
First crop, second year	.33	.44	.38	.39	.26	.32	.37	.50	.44	.81	.58	.70	.46
Second crop, second year	.41	.48	.44	.36	.31	.34	.29	.30	.30	.72	.52	.62	.42

A question of considerable interest is whether the individual roots increase or decrease in weight the second year. A loss in the total weight of roots per acre does not necessarily indicate a loss in weight of the individual roots since such total loss might be caused by a decrease in stand due to natural competition of the plants. Furthermore, as Willard (14, p. 38) states:

* * * the plants that die weigh much less than those that remain alive and the latter would therefore apparently increase in weight whether they actually grew or not.

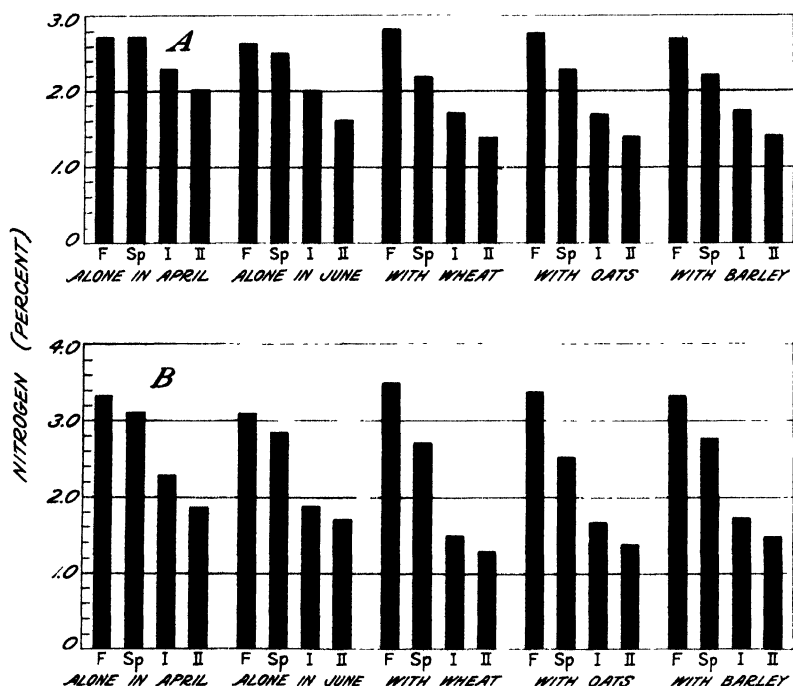


FIGURE 3.—Percentage of nitrogen in the roots of Albotrea (A) and biennial white (B) sweetclover at various periods and when sown with and without companion crops. F, October, first year, Sp, May second year, I, first hay crop; II, second hay crop.

The average weights of roots from single plants are given in table 6. These were obtained by dividing the total weights of roots per square yard by the number of plants. Of 40 such samples, 25 showed a decrease in weight of individual roots from October of the first year to July of the second year, 14 showed an increase, and 1 was the same. In 5 of the 25 samples showing a decrease in weight, the number of plants in July was greater than in October so that such apparent decrease could have resulted from the increased number. By the method of dividing the total weight by the number of plants an increased number would naturally tend to show a decrease in the weights of the individual roots. In all other samples there was a lower number of plants in July than in October of the first year.

PERCENTAGE OF NITROGEN IN TOPS AND ROOTS

The percentage of nitrogen found in the tops of the biennial white sweetclover at the first and second cuttings of hay and the percentage of nitrogen in the roots of biennial white and Albotrea at four stages

of growth are presented in table 7. Very slight variations occurred in the nitrogen content under different methods of sowing. The amount contained in the second cutting, however, was definitely lower than in the first.

In figure 3, the nitrogen content of the roots of Alborea and biennial white is illustrated at 4 harvests and under 5 methods of sowing. The decrease in nitrogen content from October of the first year to the second cutting of hay is pronounced and fairly uniform for all methods of sowing. In table 7, the acre yields of nitrogen have been computed. Although the percentage of nitrogen did not vary greatly between different methods of sowing, the acre yield of nitrogen shows important differences.

TABLE 7.—*Percentage of nitrogen and pounds of nitrogen per acre in tops and roots of biennial white and in roots of Alborea at 4 stages of growth, when sown alone early and alone late, and with companion crops*

Method of sowing	Biennial white tops		Biennial white roots				Alborea roots			
	First hay crop	Second hay crop	October, first year	May, second year	First hay crop	Second hay crop	October, first year	May, second year	First hay crop	Second hay crop
Alone in April.....	3 12	2 22	3 30	3 11	2 24	1 87	2 71	2 69	2 25	1 99
Alone in June.....	3 20	2 13	3 06	2 82	1 86	1 71	2 61	2 53	1 97	1 61
With wheat.....	3 11	2 21	3 44	2 68	1 56	1 30	2 80	2 18	1 66	1 39
With oats.....	3 27	2 36	3 37	2 57	1 66	1 39	2 76	2 26	1 69	1 40
With barley.....	3 12	2 26	3 26	2 74	1 71	1 52	2 64	2 21	1 68	1 40
Average.....	3 16	2 24	3 29	2 78	1 81	1 56	2 70	2 37	1 85	1 56

POUNDS OF NITROGEN PER ACRE										
Alone in April.....	45 5	35 0	83 6	44.5	35 7	32 6	96.2	49.8	41.2	28.6
Alone in June.....	40 3	49 2	39 4	20 8	16 0	14 3	52 5	29 2	21 7	15.3
With wheat.....	57 0	55.9	42 0	21 6	15 3	10 2	48 8	23 2	17 0	12.1
With oats.....	36.4	44.7	26 9	13 1	12 7	10 2	27 2	14 6	14 0	7 9
With barley.....	33 2	46.4	28.5	16 3	13.2	10 5	36 8	18 9	14.5	10.2
Average.....	42 5	48 2	44 1	23 3	18.6	15 6	52 3	27.1	21.7	14.8

DISCUSSION

This investigation was conducted under favorable conditions. The soil contained sufficient lime for successful growth of sweetclover. It represented a type intermediate between the heavy and light soils of the valley and was in a good state of fertility. The seasons of 1931 and 1932 were drier and warmer than the 10-year average, but under the drainage conditions of the experimental farm such seasons are more favorable than abnormally wet years.

Ceres wheat, Anthony oats, and Trebi barley, high-yielding varieties extensively used in the valley, were chosen for the companion crops. There was no lodging of any grain crop so this factor did not enter into the comparison. However, the apparent definite superiority of wheat as a companion crop for sweetclover may have been due to the variety of oats used. It is possible that an early, short-growing oat would have proved better as a companion crop than Anthony which is mid-season and mid-tall. On the other hand, farmers select grain crop varieties primarily for yield and only secondarily as companion crops.

The June seeding was handicapped by weeds which generally prevail in sowing without companion crops, although the ground was kept cultivated until seeding time. The early seeding grew fast enough

to keep the weeds in better control. For root development there appears to be a decided advantage in sowing alone early. For maximum yield of roots such seeding is definitely superior to the use of a companion crop not only during the first year but continuing throughout the second season. The practicability of sowing alone, however, is open to question, since the loss of the grain crop is entailed and weeds are not kept so well under control.

It is evident from the data that some sweetclover seeds sown with the grain crops did not germinate until after the companion crop was removed since in every instance there were more plants per square yard in October of the first year than at the time of grain harvest. Apparently the stands obtained by all methods of sowing were adequate under the favorable conditions of the trial, since no significant relationship existed between stand the first year and subsequent yields. Moreover, the ultimate stand was in all cases much larger than that found by Willard (14). An analysis of 15 comparisons of stand showed (1) that in only 3 instances a thicker stand resulted in a better yield of roots and tops, (2) that in no instance did a decrease in stand result in either a better or poorer yield, and (3) that in 12 instances there was no significant relationship of any kind.

In yields of hay, the biennial white ranked first, Albotrea last, and the biennial yellow and Grundy County about equal and in an intermediate position. Choice of variety for a hay crop, however, might depend on other factors than yield.

Whether the individual roots increase or decrease in weight from late fall of the first year to July of the second year is not clear, although the data appear to show a decrease. The possibilities of drawing conclusions from the data obtained may be stated as follows:

(1) If an increase in stand is accompanied by a loss in weight of the individual roots, no conclusion concerning this question is justified since the total weight of the roots per square yard divided by an increased number would tend to show a loss in weight.

(2) If loss in stand is accompanied by an increase in weight, no conclusion is justified since the plants that died were presumably of less weight than those that lived and hence would tend to show an increase in weight of the latter.

(3) If an increase in stand is accompanied by an increase in weight, the conclusion is justified that the individual roots increased in weight.

(4) If a loss in stand is accompanied by a loss in weight, the conclusion is justified that the individual roots actually lost in weight.

An examination of 40 comparisons using these possibilities of conclusions showed a loss of weight in 20 and no justifiable conclusion in the remaining 20.

Grundy County proved inferior to the other kinds of sweetclover in root development. For plowing under in the fall of the first year or early spring of the second year, the two yellow kinds were superior to the white since they made both a significantly larger root development and in 3 comparisons out of 4 a significantly larger total weight of tops, stubble, and roots than the white. In the second year, however, this did not hold true of the total weight, and root production was about the same. Albotrea was superior to the biennial yellow as a crop for plowing under in the fall of the first year or early spring of the second year, and biennial white was definitely better than Grundy County for plowing under in either year.

The maximum root development and nitrogen content occurred without exception in the late fall of the first year. The maximum total weight occurred at the time of the first or second hay crop when sown with a companion crop or alone in June. When the sweetclover was sown alone in April, this occurred in October of the first year, with the exception of Grundy County. No nitrogen determinations were made for tops in the fall. If it is assumed that the percentage of nitrogen is the same as in the first hay crop, as Kirk (?) found, the maximum total nitrogen per acre in both tops and roots of biennial white occurred in October of the first year.

As far as total weight and nitrogen per acre in this investigation are concerned, the best time to plow under sweetclover for soil improvement is in the late fall of the first year and the best method of sowing is early without a companion crop. However, other factors not investigated in these trials are important. It is very difficult to kill sweetclover in the fall of the first year. Plowing under the maximum amount of organic matter may not prove most successful for succeeding crops. Weeds may make sowing sweetclover alone impracticable even though seeded early, and the loss of the grain crop is an economic factor. The next best method, again considering total weight and nitrogen per acre, is to sow with wheat and plow under either the first or second crop. Again other factors not investigated are present. Plowing under the first crop leaves too short a season in the valley for planting most other crops and a long season for fallowing. Furthermore, plowing under the maximum amount of organic matter may not prove the best method. Investigations in the methods of handling biennial white sweetclover for soil improvement under the supervision of the soils division, University Farm, are now under way at Crookston.

SUMMARY

Two types of sweetclover, biennial white (*Melilotus alba*), biennial yellow (*Melilotus officinalis*), and a variety of each, Grundy County and Albotrea, were grown at the Northwest Experiment Station, Crookston, Minn., in 1931 and 1932. A comparison was made of yield of roots and tops of the four types at five stages of growth, sown with and without companion crops. The nitrogen content of tops and roots of biennial white and Albotrea was studied.

Larger yields of tops were obtained from all four kinds of sweetclover when sown with Ceres wheat, than with Anthony oats or Trebi barley. With the exception of Grundy County, all sweetclovers sown alone in April produced more top growth by October of the first year than in any harvest the second year under the same method of sowing. Grundy County, however, yielded most in the second hay crop the second year.

The maximum yield of roots was produced by each sweetclover when sown alone in April. When sown with companion crops, the largest yield of roots was produced from the sweetclover sown with wheat, and the least from that sown with oats.

When the sweetclovers were sown alone in April the maximum total weight of roots, tops, and stubble occurred in October of the first year for all except Grundy County. The latter made its maximum development at the time of cutting the first hay crop of the second year.

When sown with a grain crop or alone in June, however, the maximum total weight was produced in the second year, 12 times at the second hay crop and 4 times at the first.

Stands were not greatly affected by the type of companion crop used. The stand increased when sown with companion crops from the time the grain was removed until October of the first year. In every comparison but two the stand decreased from October of the first year to July of the second. The average decrease for all sweetclovers was: 34.77 percent sown with wheat; 36.61 percent with oats; 25.49 percent with barley; 22.71 percent sown alone in April; and 35.45 percent sown alone in June.

Grundy County gave a lower yield of roots than did the other three varieties. Differences in yield of tops for the other three varieties were not significant.

In the first year the two yellow sweetclovers produced larger total yields of roots, stubble, and tops than the white in 3 out of 4 comparisons, but this did not hold true for the second year. When sown alone in April, Alborea and biennial white produced the largest total weights of all types and varieties in October of the first year.

Without any exception, the maximum weight of roots was found in October of the first year. In every instance the weight of roots was less the following May. Individual roots appear to decrease in weight from October of the first year to July of the second season.

The nitrogen percentage in the tops of biennial white was lower in the second hay crop than in the first and was not greatly influenced by the different methods of sowing. The nitrogen percentage in the roots was much lower in July of the second season than in October of the first year.

The maximum total nitrogen per acre in both tops and roots of biennial white was produced in October of the first year.

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TEMPERATURE RELATIONS OF WOOD-DESTROYING FUNGI¹

By C. J. HUMPHREY, formerly *pathologist*, and P. V. SIGGERS, *associate pathologist*, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture²

INTRODUCTION

A knowledge of the rate of growth of wood-destroying fungi at different temperatures is of considerable importance to the pathologist working on problems of timber decay. In fact, the effectiveness of control measures may at times be largely dependent upon the behavior of the organism under the temperature conditions imposed. This is particularly true of certain dry-rot fungi whose development may be arrested by raising, as far as practicable, the temperature of the air in buildings.

The present paper records the growth, on malt-extract agars in Petri dishes, of 56 named species and several strains of fungi, most of which are commonly found in various parts of the United States and Cuba, and some of which are cosmopolitan in their distribution. A considerable number that are of great economic importance are represented on account of the severe decay which they cause in structural timber.

MATERIALS AND METHODS

Two different media were used, each of which contained malt extract. Medium 1 had the following composition: Distilled water, 1,000 cc; beef extract (Armour's), 5 g; Löfflund's malt extract, 25 g; agar-agar, in strips, 20 g. The mixture was carefully filtered, but the reaction was not adjusted and remained somewhat acid. This is a very good medium for the development of wood-destroying fungi.

Medium 2 consisted of distilled water, 1,000 cc; Trommer's malt extract (plain), 25 g; agar-agar (Bacto, granulated), 20 g. The mixture was filtered, but the reaction was not adjusted (+ 8.5 to + 10.5, Fuller's scale).

Seven liters of medium 1 was prepared at one time. The agar was dissolved in several 2-l Erlenmeyer flasks by adding the desired amount of water and cooking in an autoclave at 100° C. for about 1½ hours. In order to make the medium as uniform as possible the different lots were then mixed in an enamel-ware pan. To the pan of hot solution were added the necessary amounts of beef extract and malt extract, which were then thoroughly mixed with the agar. The medium was then filtered through cotton, tubed in 20-cc quantities, and sterilized in the autoclave for 40 minutes at a pressure of 15 pounds.

Eighteen liters of medium 2 was made at one time. This was done by placing the Bacto agar in 2-l Erlenmeyer flasks containing 1,500 cc of distilled water and heating for 2½ hours in the autoclave at 100°

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² The writers wish to express their appreciation to Mrs. Ruth M. Fleming for much assistance during the early part of the investigations reported herein.

C. The separate quantities were then poured into a large enamel-ware pan, which was kept warm by a Bunsen burner until the malt extract had been added, and the medium was then filtered through cotton into 1,000-cc Erlenmeyer flasks. Without allowing the agar to harden, the flasks were sterilized in the autoclave for 30 minutes without pressure. On each of the two following days they were again heated for 1½ hours without pressure. After cooling they were placed in the ice box for use as needed.

Whenever tests were started the necessary number of flasks were steamed for an hour at 100° C., and then the contents were tubed in 20-cc quantities and sterilized for 30 minutes without pressure on each of 3 successive days. On the third day the contents of the tubes were poured while hot into sterile Petri dishes.

The Petri dishes used in the tests were 100 mm in diameter and 12 to 15 mm deep. The lids were carefully selected so as to secure a close fit and avoid unnecessary drying of the medium. After it was poured, the agar was inoculated at the center of the plates by depositing a square of fungus growth, usually 8 by 10 mm in size, but occasionally smaller, that had been cut from near the margin of another Petri-dish culture and was therefore in a vigorous growing condition.

The cultures from which these squares were taken differed somewhat in age. Those used for medium 1 (first series) usually ranged from 13 to 22 days and were rarely 60 days old; those used for medium 2 (second series) ranged from 11 to 25 days and were occasionally 31 days old. As far as could be determined, however, the difference in age had no apparent influence on the rate of growth. This was probably due to the fact that the squares of inoculum were cut from the growing margin or near it.

After inoculation, the dishes were stacked in electric or gas-heated incubators maintained at the desired temperature by thermostatic control. For temperatures above that of the air in the room a water-jacketed electric incubator was usually employed; for lower temperatures a gas-heated Hearson incubator was used. The variation of temperature in these incubators at the points where the thermometers were inserted was usually about 0.5° C. The variation was probably greater between widely separated points in the chamber, because the heavy loading impeded air circulation and the application of heat at the bottom of the electric incubators tended to raise the temperature at that point above the desired limit. However, crowding of the cultures ceased as a factor in temperature variation from 38° to 46°, since at these temperatures heavy loading never occurred. The Hearson incubators were more uniform in temperature within, since in these the temperature is controlled automatically by adding small quantities of hot or cold water to the jacket as needed.

The thermometers used were checked against a standardized instrument. During tests at the different temperatures in the second series (on medium 2) the averages of all the readings recorded in no case varied more than 0.6° C. from the desired temperatures.

Radial growth in millimeters from the edge of the inoculum was recorded at the end of the first and second weeks. Measurements were made with a thin millimeter scale held against the inverted dish. Since growth was not always uniform on all sides of the inoculum, the average was computed as nearly as possible.

In the first series (on medium 1) the plates were made in duplicate, and the value given for rate of growth represents the average for the two plates. In the second series (on medium 2) the cultures were prepared and averaged in triplicate.

An alphabetical tabulation of species and cultures studied, together with their sources and an index of the graphs, is given in table 1. The stock cultures used were obtained by the senior writer and were mostly his own isolations..

TABLE 1.—List of species and cultures with their sources and localities, and an index of the graphs

Fungus	Source	Locality	Fig. no.
<i>Collybia velutipes</i> (Curt.) Fr.	Spores	Wisconsin	1, C
<i>Coniophora cerebella</i> Pers., no. 1	do.	Europe	1, D
<i>Coniophora cerebella</i> Pers., no. 2	Spores	Illinois	2, G
<i>Corticium chrysocreas</i> Berk. and Curt.	do.	Louisiana	2, G
<i>Corticium effusatum</i> Cke. and Ell.	do.	Illinois	2, L
<i>Daedalea ambigua</i> Berk.	do.	Mississippi	2, Q
<i>Daedalea quercina</i> (L.) Fr.	Sporophores	Massachusetts	3, K
<i>Daedalea unicolor</i> (Bull.) Fr.	Spores	Wisconsin	2, P
<i>Fomes annosus</i> (Fr.) Cke., no. 1	do.	Europe	1, E
<i>Fomes annosus</i> (Fr.) Cke., no. 2	Sporophores	Pennsylvania	3, L
<i>Fomes ererhartii</i> Ell. and Gull.	do.	Wisconsin	2, R
<i>Fomes ignarius</i> (L.) Gull.	do.	Montana	3, E
<i>Fomes marmoratus</i> (Berk. and Curt.) Cke.	do.	Florida	2, S
<i>Fomes nigrolutatus</i> Ronell.	do.	Washington	1, F
<i>Fomes officinalis</i> Fr.	do.	do.	1, J
<i>Fomes pinicola</i> (Swendener) Cke.	do.	Montana	1, V
<i>Fomes rimosus</i> Berk.	do.	Ohio	2, T
<i>Fomes subroseus</i> Weir.	do.	United States	2, E
<i>Ganoderma applanatum</i> (Pers.) Pat.	do.	Indiana	1, I
<i>Ganoderma lucidum</i> (Leyss.) Karst.	do.	Wisconsin	3, R
<i>Hydnum ochraceum</i> (Pers.) Fr.	Spores	do.	1, N
<i>Hydnum pulcherrimum</i> Berk. and Curt.	do.	Louisiana	3, G
<i>Irpex mollis</i> Berk. and Curt.	do.	Wisconsin	3, H
<i>Lentinus lepideus</i> Fr., no. 1	do. (?)	Montana	2, C
<i>Lentinus lepideus</i> Fr., no. 2	do.	Wisconsin	3, B
<i>Lenzites berkeleyi</i> Lév.	Sporophores	Florida	3, M
<i>Lenzites sarcinaria</i> (Wulf.) Fr.	Spores	Wisconsin	2, F
<i>Lenzites striata</i> (Sw.) Fr.	do.	Cuba	3, N
<i>Lenzites trabea</i> (Pers.) Fr., no. 1	do.	Wisconsin	3, O
<i>Lenzites trabea</i> (Pers.) Fr., no. 2	do.	Illinois	3, P
<i>Merulius himantoides</i> Fr.	do.	Wisconsin	2, A
<i>Merulius lacrymans</i> (Wulf.) Fr., no. 1	do.	Europe	1, B
<i>Merulius lacrymans</i> (Wulf.) Fr., no. 2	Building timber	Rhode Island	1, A
<i>Merulius silvester</i> Falck	do.	Europe	1, K
<i>Merulius tremellosus</i> (Schrud.) Fr.	Spores	Florida	3, I
<i>Panus rudis</i> Fr.	do.	Minnesota	3, S
<i>Peniophora gigantea</i> (Fr.) Mass.	do.	Michigan	1, T
<i>Phlebia merismoides</i> Fr.	do.	do.	1, Q
<i>Phlebia strigosozonata</i> (Schw.) Lloyd	Decayed wood	Minnesota	2, V
<i>Pleurotus ostreatus</i> (Jacq.) Fr.	Spores	Wisconsin	2, U
<i>Polyporus maximus</i> (Mont.) Overh.	do.	Cuba	2, K
<i>Polyporus radiatus</i> (Sow.) Fr.	Sporophores	Wisconsin	1, L
<i>Polyporus robinophilus</i> (Murr.) Lloyd	do.	Ohio	2, J
<i>Polyporus schweinitzii</i> Fr.	do.	Florida	1, M
<i>Polyporus sulphureus</i> (Bull.) Fr.	do.	Montana	2, D
<i>Polystictus abietinus</i> (Dicks.) Fr. (<i>Irpex fusco-roseaceus</i> (Schrud.) Fr.)	Spores	New York	1, O
<i>Polystictus abietinus</i> (Dicks.) Fr.	Decayed wood	Wisconsin	2, H
<i>Polystictus hirsutus</i> (Wulf.) Fr.	Spores (?)	do.	3, I
<i>Polystictus sinuosus</i> Fr.	Spores	New York	3, I
<i>Polystictus tenuis</i> (Lk.) Cke.	do.	Cuba	3, A
<i>Polystictus versicolor</i> (L.) Fr.	Sporophores	Wisconsin	2, J
<i>Poria incrassata</i> (Berk. and Curt.) Burt.	Building timber	Mississippi	1, R
<i>Poria subacida</i> (Pk.) Sacc.	Spores	Michigan	1, P
<i>Poria xantha</i> Fr. sensu Lind.	do.	Montana	1, S
<i>Schizophyllum commune</i> Fr., no. 1	do.	Wisconsin	3, D
<i>Schizophyllum commune</i> Fr., no. 2	do.	Cuba	3, C
<i>Stereum fasciatum</i> Schw.	do.	Wisconsin	1, U
<i>Stereum frustulosum</i> (Pers.) Fr.	do.	do.	3, J
<i>Stereum fusum</i> (Schrud.) Quel.	do.	Florida	1, C
<i>Stereum gausapatum</i> Fr.	do.	Wisconsin	2, M
<i>Stereum ramale</i> Schw.	do.	do.	1, W
<i>Trametes pini</i> (Thore)	Decayed wood	Idaho	1, H
<i>Trametes serialis</i> Fr., no. 1	Sporophores	Wisconsin	2, B
<i>Trametes serialis</i> Fr., no. 2	Spores	do.	1, G

* Culture from Dr. Westerdijk, Netherlands.

† *Merulius silvester* is considered synonymous with *M. himantoides*.

EXPERIMENTAL DATA

In order to present the growth data in a form easily comprehensible, graphs (figs. 1-3) have been prepared for each of the 64 species or strains studied. The general arrangement of the graphs is in the order of increasing maximum temperatures at which growth ceases;

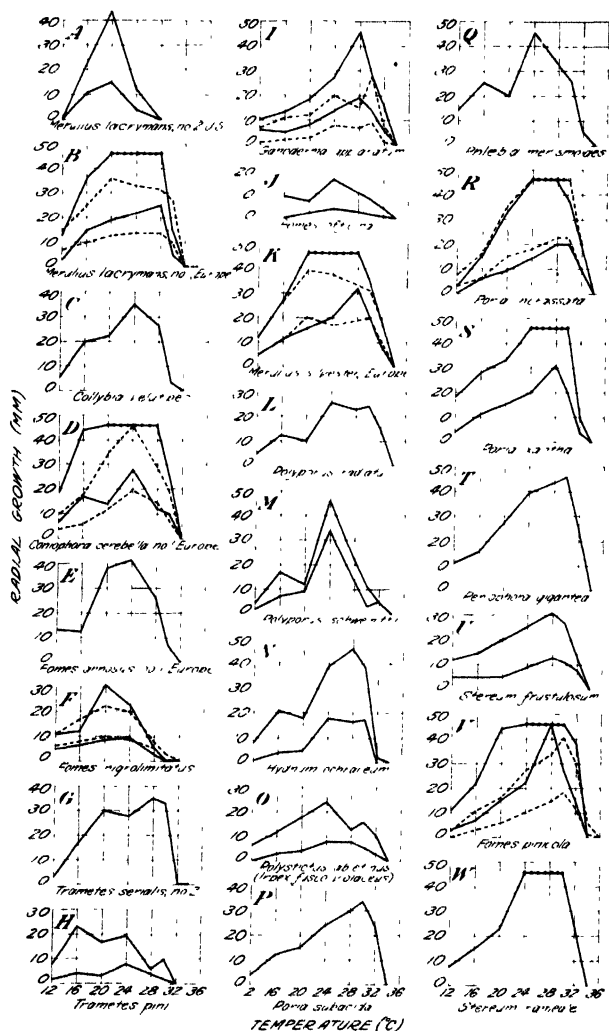


FIGURE 1—Graphs (A-W) showing growth of 23 wood-destrating fungi at different temperatures. A solid line represents 1 week's growth on medium 1; a broken line represents 1 week's growth on medium 2. Where two solid lines or two broken lines are shown, the upper represents 2 weeks' growth. A dotted line indicates that the fungus grew to the edge of the Petri dish before the first measurement was made.

thus the graphs with the lowest maxima are grouped on figure 1, and those with the highest maxima appear on figure 3. Since the rate of growth during the second week may present a smoother curve than that during the first week, on account of the equalization of slight irregularities that occurred in the development of the fungus

during the first 2 or 3 days while it was becoming established, the data for both 1 and 2 weeks are plotted wherever available. The data for the second week, however, would have been of no significance in the case of certain species which grew so rapidly that they covered the

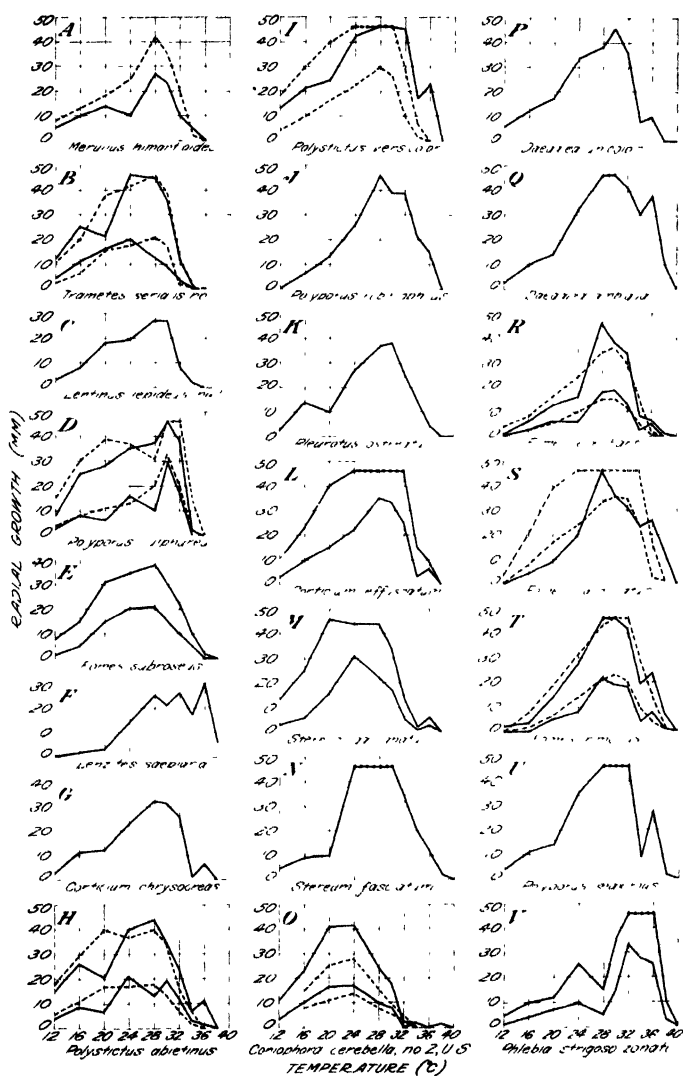


FIGURE 2.—Graphs (A-V) showing growth of 22 wood-destroying fungi at different temperatures. A solid line represents 1 week's growth on medium 1; a broken line represents 1 week's growth on medium 2. Where two solid lines or two broken lines are shown, the upper represents 2 weeks' growth. A dotted line indicates that the fungus grew to the edge of the Petri dish before the first measurement was made.

plates during the first week over a considerable temperature range. Had larger dishes been used or had measurements been taken at shorter intervals, the rate of growth could have been more carefully checked and the optimum temperature for the rapidly growing species could, in many instances, have been more closely defined.

In some of the later work with medium 2, an attempt was made to provide a larger radius for growth by planting the inoculum on one side of the dish in the case of rapidly developing species. In other instances the short period required to cover the dish was recorded

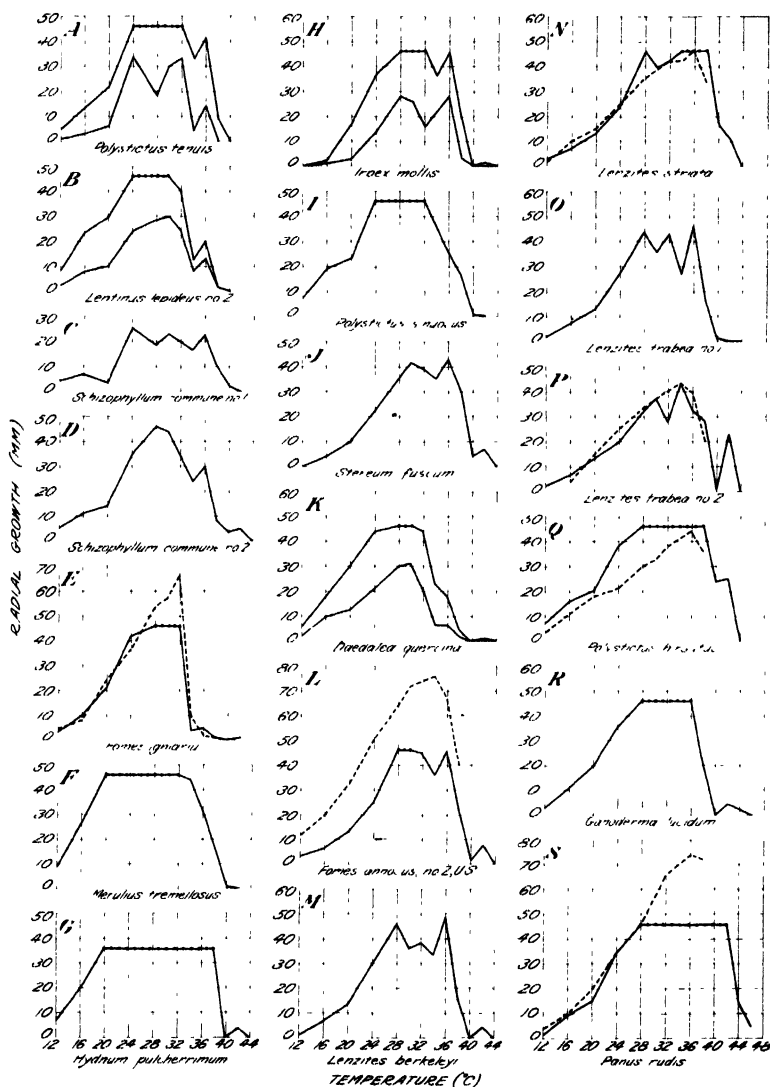


FIGURE 3.—Graphs (A-S) showing growth of 10 wood-destrating fungi at different temperatures. A solid line represents 1 week's growth on medium 1, a broken line represents 1 week's growth on medium 2. Where two solid lines or two broken lines are shown, the upper represents 2 weeks' growth. A dotted line indicates that the fungus grew to the edge of the Petri dish before the first measurement was made.

and the total growth for the week was computed on the assumption that the rate of growth was constant. There are possible errors in both methods. An inhibition of growth on one side of the inoculum, due to proximity to the side of the dish, may possibly result in stronger

growth on the free side, and it cannot safely be assumed in either case that a uniform rate of growth is maintained from the beginning. In fact, as a number of the graphs indicate, acceleration in growth occurred during the second week of observation. Monteith and Dahl,³ working with nine strains of *Rhizoctonia solani* Kuehn, also found a difference in the rapidity with which new cultures started growth, even when transfers were taken from corresponding segments of mycelium from the same plate; however, when once established they grew at nearly the same rate.

While the plotted data indicate in most cases a fairly regular progression from the less favorable temperatures to the optimum, there are a number of apparent discrepancies. Some of the cultures became contaminated or the dishes were broken, leaving only one for measurement, as indicated in table 2. On medium 1 certain cultures showed considerably more rapid growth in one dish than in the other, while on medium 2 there may have been a rather wide variation for the three dishes. All serious disagreements between duplicates are also shown in table 2.

TABLE 2.—Comparison of duplicate cultures showing difference in growth and instances where only one dish was available for measurement at the end of the first or the second week

Medium and fungus	Temperature	Radial growth of duplicate cultures for the first week		Medium and fungus	Temperature	Radial growth of duplicate cultures for the first week	
MEDIUM 1				MEDIUM 1—continued			
	°C.	Mm	Mm		°C.	Mm	Mm
<i>Fomes annosus</i> (Europe).....	16	9	16.5	<i>Irpep mollis</i>	24	(a)	(b)
<i>Stereum gausapatum</i>		(a)	—	<i>Polyporus sulphureus</i>		(b)	(b)
<i>Collybia velutipes</i>		4	22.5	<i>Poria rantha</i> (sensu Lind).....		(b)	(b)
<i>Daedalea ambigua</i>		10	17.5	<i>Conophora cerebella</i> (Europe).....		11.5	14
<i>Fomes marmoratus</i>		4	15	<i>Ganoderma applanatum</i>		17	21
<i>Hydnum pulcherrimum</i>	20	(a)	—	<i>Polystictus abietinus</i> (<i>Irpep fusco-olivaceus</i>).....	28	(a)	—
<i>Polystictus abietinus</i> (<i>Irpep fusco-olivaceus</i>).....		(a)	—	<i>Polystictus abietinus</i>		(a)	—
<i>Polystictus sinuosus</i>		20	27	<i>Polyporus sulphureus</i>		35	45
<i>Lentinus lepidus</i> , no. 2.....		5	15	<i>Stereum rameale</i>		(a)	—
<i>Lenzites berkeleyi</i>		9	18	<i>Trametes pini</i>		(a)	—
<i>Lenzites striata</i>		11	15	<i>Daedalea unicolor</i>	32	(a)	—
<i>Merulius lacrymans</i> (United States).....		13	17	<i>Irpep mollis</i>		(a)	—
<i>Phlebia merismoides</i>		12.5	27.5	<i>Phlebia merismoides</i>		(a)	—
<i>Pleurotus ostreatus</i>		8	13.5	<i>Polyporus maximus</i>		(a)	—
<i>Polystictus hirsutus</i>		17	24	<i>Irpep mollis</i>	34	(a)	—
<i>Polyporus radiatus</i>	20	3	12.5	<i>Merulius tremellosus</i>		(a)	—
<i>Polyporus schweinitzii</i>		8	15	<i>Lenzites berkeleyi</i>		(a)	—
<i>Polystictus versicolor</i>		21.5	26	<i>Stereum fuscum</i>		(a)	—
<i>Schizophyllum commune</i> , no. 1.....		(a)	—				
<i>Stereum rameale</i>	20	20	27.5	MEDIUM 2			
<i>Trametes pini</i>		1.5	5.5	<i>Merulius lacrymans</i> (Europe).....	28	(b)	—

a Only 1 dish available for measurement at end of first week.

b Only 1 dish available for measurement at end of second week.

c Growth at 2 weeks.

The variations for the 20° and 28° cultures on medium 1 can perhaps be explained by the fact that the medium for one of the pair had a suggestion of yeast growth in the stock flask which necessitated a resterilization for 45 minutes at a pressure of 15 pounds. The agar would not then solidify as firmly as before, and its composition was

³ MONTEITH, J., Jr., and DAHL, A. S. A COMPARISON OF SOME STRAINS OF RHIZOCTONIA SOLANI IN CULTURE. Jour. Agr. Research 36: 897-903, illus. 1928.

probably somewhat changed. Discrepancies occurred frequently at the upper temperature limits and may perhaps have been partly due to variations in temperature in different parts of the incubator chamber, for it is probable that the variation would be great where considerable heat was applied almost continuously at the bottom, as was done in all the tests run at temperatures near the maximum inhibition point for the more resistant species. Furthermore, as the critical point is approached a slight change in temperature may produce a profound effect on both the rate and character of growth. For this reason it has not always been possible to closely define the maximum inhibition point.

It is possible, though not very probable, that the exposure to daylight necessary for recording growth measurements at the end of the first week may, in certain instances, explain the reduction in growth rate. Fritz,⁴ for instance, found daylight inhibitive to the development of *Polyporus schweinitzii* in culture.

The species may be arranged in three general temperature groups: (1) A low-temperature group, growing best at 24° C. and below; (2) an intermediate group, growing best between 24° and 32°; and (3) a high-temperature group, growing best above 32°.

In table 3 the tested fungi are arranged according to their most favorable temperatures for growth on medium 1, starting at the lowest temperature. The optima for medium 2 are given in comparison wherever available, although the order may be different from that for medium 1. The optima are for the most part based on 1 or 2 weeks' growth. The maximum temperature at which growth ceases and the difference between it and the optimum for the different species and cultures are also indicated.

TABLE 3.—Optimum temperatures for growth and the maximum temperatures which prevent growth of the tested fungi

LOW-TEMPERATURE GROUP (OPTIMUM 24° C. AND BELOW)

Fungus	Temperature (° C.) affecting growth of fungi on—					
	Medium 1			Medium 2		
	Opti- mum	Inhib- iting	Differ- ence	Opti- mum	Inhib- iting	Differ- ence
<i>Merulius lacrymans</i> (United States).....	20	28	8			
<i>Fomes nigrolimitatus</i>	20	32	12	20	32	12
<i>Trametes pini</i>	20	32	12			
<i>Coniophora cerebella</i> (Europe).....	24	32	8	24	32	8
<i>Fomes annosus</i> (Europe).....	24	32	8			
<i>Collybia velutipes</i>	24	32	8			
<i>Coniophora cerebella</i> (United States).....	24	34	10	24	34	10
<i>Fomes officinalis</i>	24	34	10			
<i>Phlebia merismoides</i>	24	34	10			
<i>Polyporus schweinitzii</i>	24	34	10			
<i>Polystictus abietinus</i> (<i>Irpex fusco-violaceus</i>).....	24	34	10			
<i>Stereum gausapatum</i>	24	38	14			

* In order to establish an optimum where it is not clearly defined by the graphs, it has been necessary to take into consideration obvious defects in the experimentation and to make as close an estimate as possible from the data at hand.

^b Trace of growth noted after the second, third, or fourth week.

^c Trace of growth at 38° C.

⁴ FRITZ, C. W. CULTURAL CRITERIA FOR THE DISTINCTION OF WOOD-DESTROYING FUNGI. Roy. Soc. Canada, Proc. and Trans. (3) 17 (sec. 5): 191-288, illus. 1923.

TABLE 3.—Optimum temperatures for growth and the maximum temperatures which prevent growth of the tested fungi—Continued

INTERMEDIATE-TEMPERATURE GROUP (OPTIMUM BETWEEN 24° AND 32° C.)

Fungus	Temperature (° C.) affecting growth of fungi on—					
	Medium 1			Medium 2		
	Opti- mum	Inhib- iting	Differ- ence	Opti- mum	Inhib- iting	Differ- ence
<i>Trametes serialis</i> , no. 1	24	34	10	28	36	8
<i>Merulius lacrymans</i> (Europe)	^a 26	^b 32	6	^a 24	34	10
<i>Merulius silvester</i> (Europe)	^a 26	34	8	^a 24	34	10
<i>Ganoderma applanatum</i>	28	34	6	^a 28	34	6
<i>Hydnum ochraceum</i>	28	34	6			
<i>Poria incrassata</i>	^a 28	34	6	^a 28	^b 34	
<i>Stereum rameale</i>	^a 28	34	6			
<i>Trametes serialis</i> , no. 2	28	34	6			
<i>Poria zantha</i> (sensu Lind)	^a 28	34	6			
<i>Polyporus radiatus</i>	^a 28	34	6			
<i>Stereum frustulosum</i>	^a 28	34	6			
<i>Lentinus lepideus</i> , no. 1	^a 28	36	8			
<i>Merulius himantoides</i>	28	36	8	28	36	8
<i>Polystictus abietinus</i>	28	38	10	^a 28	36	10
<i>Polystictus versicolor</i>	^a 28	38	10	28	36	8
<i>Corticium chrysocreas</i>	28	38	10			
<i>Corticium effuscatum</i>	28	38	10			
<i>Fomes subrostratus</i>	28	38	10			
<i>Polyporus robinophilus</i>	28	38	10			
<i>Lentinus lepideus</i> , no. 2	^a 28	40	12			
<i>Stereum fasciatum</i>	^a 28	40	12			
<i>Fomes pinicola</i>	28	34	6	30	36	6
<i>Fomes everhartii</i>	28	40	12	30	38	8
<i>Fomes marmoratus</i>	28	40	12	^a 30	38	8
<i>Merulius tremellosus</i>	^a 28	42	14			
<i>Polystictus sinuosus</i>	^a 28	42	14			
<i>Schizophyllum commune</i> , no. 2	28	44	16			
<i>Peniophora gigantea</i>	30	34	4			
<i>Poria subacida</i>	30	34	4			
<i>Polyporus sulphureus</i>	30	36	6	30	36	6
<i>Daedalea ambigua</i>	^a 30	40	10			
<i>Daedalea unicolor</i>	30	40	10			
<i>Fomes rimosus</i>	^a 30	40	10	30	40	10
<i>Pleurotus ostreatus</i>	30	40	10			
<i>Polystictus tenuis</i>	^a 30	40	10			
<i>Polyporus maximus</i>	^a 30	40	10			
<i>Schizophyllum commune</i> , no. 1	^a 30	42	12			
<i>Fomes ignarius</i>	^a 30	42	12	32	40	8
<i>Daedalea quercina</i>	^a 30	44	14			
<i>Irpex mollis</i>	^a 30	44	14			
<i>Lenzites berkeleyi</i>	^a 32	44	12			
<i>Hydnum pulcherrimum</i>	^a 32	44	12			

HIGH-TEMPERATURE GROUP (OPTIMUM ABOVE 32° C.)

<i>Phlebia strigoso-zonata</i>	^a 34	40	6			
<i>Fomes annosus</i> (United States)	^a 34	44	10	34		
<i>Lenzites trabea</i> , no. 1	^a 34	44	10			
<i>Stereum fuscum</i>	^a 34	44	10			
<i>Polystictus hirsutus</i>	^a 34	44	12	36		
<i>Ganoderma lucidum</i>	^a 34	^b 46	12	34		
<i>Lenzites trabea</i> , no. 2	34	^b 46	12			
<i>Lenzites striata</i>	^a 36	44	8	36		
<i>Panus rudis</i>	^a 36	(^c)	10+	36		
<i>Lenzites saepebaria</i>	36	(^c)				

^a In order to establish an optimum where it is not clearly defined by the graphs, it has been necessary to take into consideration obvious defects in the experimentation and to make as close an estimate as possible from the data at hand.

^b Trace of growth noted after the second, third, or fourth week.

^c Above 46° C.

^d Not determined.

DISCUSSION

It will be noted that the growth of about 85 percent of the species is prevented within a range of 12° above the optimum. The maximum temperature which totally inhibits growth bears no definite relationship to the optimum, but appears to be specific with the organism. However, with one exception, all the species showing a difference of 6° C. or less between the maximum and optimum temperatures have an optimum at 32° or below.

The two media gave different results, as would be expected, but these rarely differed over 2°. In some cases shifting of the maximum temperature has a physiological explanation in variation in the media. Weimer and Harter⁵ have shown that the maximum temperature which inhibits growth of *Rhizopus nigricans* Ehr. on potato agar can be raised 1° to 1.5° C. by the addition of definite amounts of dextrose.

Working with *Monilia* and *Rhizopus*, Brooks and Cooley⁶ found that *Monilia* grew just as freely at the higher temperatures and earlier and more rapidly at the lower temperatures when developed on peaches than when grown on agar, whereas the reverse condition held for *Rhizopus*. They suggested that this difference might partly be due to the fact that *Monilia* is parasitic and therefore better suited to growth on living material than is *Rhizopus*, which is saprophytic.

In a study involving four fungus parasites of citrus trees, Fawcett⁷ found that for three of the fungi the optimum temperature for growth on the same medium usually shifted to lower temperatures for each successive 24-hour observation period during culture periods of from 3 to 6 days. That this happened in certain instances is indicated by a downward shifting of the optimum temperatures in the graphs of 7 of 21 fungi by the end of the second observation period. With the other 14 cultures there was no change in the optimum temperature. There is, however, a limit to the downward shifting of the optima, for if it continued progressively, even at a reduced rate, an organism producing gradual decay, such as heart rot in standing timber, would theoretically attain its best development near the lowest temperatures that permit growth.

This downward shifting of the optimum temperatures suggests that the optima for long-time timber decay would be lower than those indicated here for comparatively short culture periods. There is, moreover, evidence that the progress of wood decay in nature is often slower than that indicated for pure cultures of fungi on agar. For instance, if the decay caused by *Polyporus schweinitzii* developed in the heartwood of a southern pine as rapidly as the fungus grew during its first week in culture at 24° C., by the end of the second year its vertical progress in the tree would be 11.6 feet. Observations on this type of decay in nature point to a much slower rate of development.

The data clearly show the marked inhibiting effect of the lowest temperatures. At 12° C. growth may be entirely inhibited or one half to two thirds retarded; with many of the species it may be

⁵ WEIMER, J. L., and HARTER, L. L. TEMPERATURE RELATIONS OF ELEVEN SPECIES OF RHIZOPUS. Jour. Agr. Research 24: 1-40, illus. 1923.

⁶ BROOKS, C., and COOLEY, J. S. TEMPERATURE RELATIONS OF STONE FRUIT FUNGI. Jour. Agr. Research 22: 451-465, illus. 1921.

⁷ FAWCETT, H. S. THE TEMPERATURE RELATIONS OF GROWTH IN CERTAIN PARASITIC FUNGI. Calif. Univ. Pubs., Agr. Sci. 4: 183-232, illus. 1921.

only 5 to 10 percent as rapid as that at the optimum. It is well known, however, that low temperatures, even those far below zero, do not kill wood-destroying fungi.

A large number of the species studied show very rapid growth over a considerable range of temperature around the optimum; these tolerate a rather high maximum, even as much as 12° to 16° C. above the optimum.

The maximum temperature which inhibits growth is of particular significance when one wishes to determine whether it will be feasible to attempt to control certain of the organisms by the artificial application of heat. Favorable results are more likely to be secured with the low-temperature group, since it is much easier to heat the timber to a point at which the organisms will be killed. The temperatures which can be applied in buildings will depend upon the effectiveness of the heating systems. In buildings having automatic sprinkler systems, the safe maximum temperature is about 46° C. (115° F.).

It has long been recognized by European investigators that the true *Merulius lacrymans* (*Merulius domesticus* Falck) is a comparatively low-temperature form (fig. 1, A and B), and this opinion is borne out by the present study. It is not unique in this respect, however, since several other species fall in the same class. *Merulius lacrymans* is not a common dry-rot fungus in the United States, although many collections of *Merulius* have been incorrectly referred to this species. So far as known, all of them grow best at temperatures higher than those most favorable for *M. lacrymans*. The growth of this fungus in Petri dishes at various temperatures is shown in figure 4.

When fruiting bodies are absent and it is desired to distinguish between the work of known and unknown fungi both of which cause the same type of decay, the data presented herein may occasionally be of service in identifying the unknown fungus by showing its temperature relationships. For example, while the rot of the American form of *Merulius lacrymans* might be confused with that of *Poria incrassata*, there is enough difference in the temperature relationships of these two fungi to permit ready differentiation in culture.

SUMMARY

In a laboratory study of the effect of temperature on the growth of 64 species and strains of wood-destroying fungi, 12 cultures were found to have optimum temperatures of 24° C. or below; 42, optimum temperatures between 24° and 32°; and 10, optimum temperatures above 32°.

The maximum temperatures for growth were determined for all but two cultures; 62 cultures stopped growth at 46° C. or below; 46 cultures, at 40° or below; and 24 cultures, at 34° or below.

With one exception, all species showing a difference of 6° C. or less between the optimum and maximum temperature had their optimum at 32° or below.

Many of the species showed a rapid growth over a considerable range of temperatures around the optimum, and these tolerated a high maximum temperature, 12° to 16° C. above the optimum.

A downward shifting of the optimum temperatures for growth for successive 7-day observation periods suggests that the

optimum temperatures for growth of wood-destroying fungi under controlled laboratory conditions are in some cases higher than

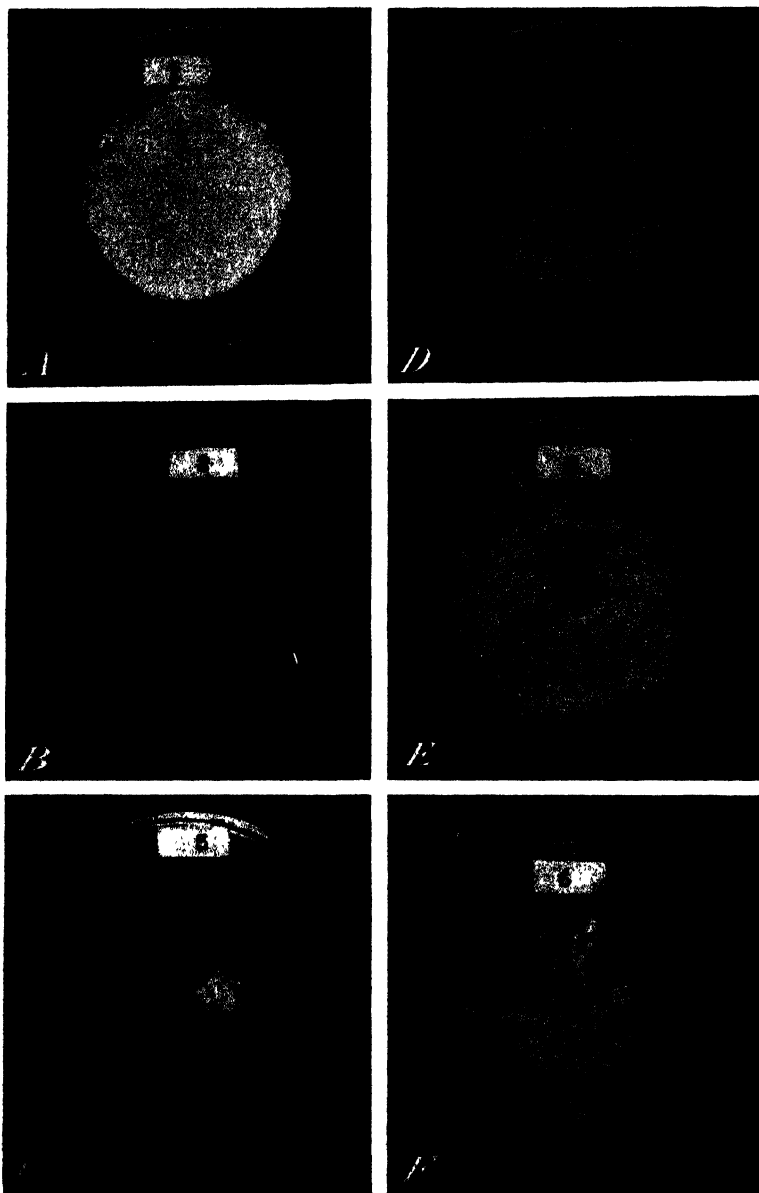


FIGURE 4.—A-C, *Merulius lacynmans* (Wulf.) Fr. (United States), growing on medium 1 (see fig. 1, A): A, 16 days old at 16° C.; B, 15 days old at 20°, its optimum; C, 32 days old at 28°. D-F, *Merulius himantioses* Fr., growing on medium 1 (see fig. 2, A): D, 16 days old at 16°; E, 16 days old at 30°; F, 31 days old at 32°.

the optima for the same decays in nature when carried over long periods.

SOME DETAILS OF MUSCLE STRUCTURE REVEALED BY SALT EXTRACTION¹

By H. N. BAKER, *assistant animal husbandman*, HUGH C. MCPHEE, *senior animal husbandman*, and PAUL E. HOWE, *senior chemist*, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

Studies have been made² of factors, in the structure of muscle, which may influence the quality of meat, especially its tenderness. Since the development of an animal involves factors of breeding, feeding, and management, it is necessary to have a technic by which variations in structure due to these factors can be determined. In a study of muscle structure two points are of particular importance: (1) the size and structure of the fibers and (2) the character and distribution of the surrounding connective tissue.

The compactness of muscle tissue often interferes with the microscopical differentiation of the elements of its structure. To facilitate the study of tissue, the writers have used various concentrations of neutral salts, such as sodium chloride, and neutral mixtures of phosphates to remove that part of the protein material which is soluble in these solvents. Examination of finely ground muscle tissue that had been extracted for the determination of the quantity of protein in fresh tissue³ indicates that although there may be some distortion of the various elements, extraction of muscle with neutral salts does not destroy the essential elements of muscle structure. Studies of extracted frozen sections indicate that the removal of the soluble proteins brings out certain details of structure that are not so evident in the unextracted material.

This paper deals especially with a technic used for the removal of proteins from muscle by sodium chloride and mixtures of monopotassium and dipotassium phosphates followed by microscopic examination of the extracted tissue.

MATERIAL AND METHODS

Muscle tissue from cattle constituted the principal source of material, although the wasp, *Polistes annularis*, was used for studying certain details of structure remaining after phosphate extraction.

Cattle tissue was taken from the muscle having its origin on the symphysis pubis as soon as the blood had drained from the body and before rigor mortis had set in. This method permitted the maximum extraction before some of the protein became denatured.

¹ Received for publication Aug. 19, 1933, issued February 1934. This study was conducted as a part of a national project, cooperative meat investigations, by the Animal Husbandry Division, Bureau of Animal Industry, U.S. Department of Agriculture, and by cooperating State agricultural experiment stations.

² BAKER, H. N., MCPHEE, H. C., and HOWE, P. E. THE MEMBRANOUS STRUCTURE OF MUSCLE. (Abstract) Anat. Rec. 41: 64, 1928.

³ See footnote 2.

The muscle usually was contracting spasmodically when the small cube of tissue was placed on the freezing microtome.

In most cases the sections were cut 25μ in thickness with a freezing microtome. Thin sections were handled by mounting on cellophane. This is accomplished by laying the cellophane on the surface of the cut section as it lies on the microtome knife (fig. 1). Such mounting facilitates the handling of the delicate sections through the various solutions and forms a mount for those sections that are carried through the usual histological procedure. It has also been of value in the further separation of the proteins from the resistant structures of the muscle.



FIGURE 1.—Longitudinal view of fiber in frozen section of beef extracted in a 0.525 molar solution of monopotassium and dipotassium phosphate for 90 minutes and fixed in mercuric chloride, mounted on cellophane, resectioned in paraffin at from 5 to 7μ , and stained with methylene blue. Note individual fibrillae within the fiber and also thickness of cellophane. $\times 800$.

Extractions of the sections of freshly killed tissue were conducted in shallow dishes (half Stender dishes) with concentrations of salt, as suggested by Howe ⁴ and the subsequent work of Ritchie and Hogan ⁵ and of Hilberg, Breden, and Howe.⁶ Analytical work by Howe ⁷ has shown that a 0.225 molar concentration of a mixture of monopotassium and dipotassium phosphates having a pH value of 7.4–7.0 gives the maximum extraction, from 40 to 60 percent of the total nitrogen, with these salts. Furthermore, a 0.525 molar concentration of the phosphates extracts practically the same quantity of protein as 0.225. Hilberg, Breden, and Howe ⁸ have found that a greater extraction of proteins from muscle is obtained with a solution of

⁴ HOWE, P. E. THE DIFFERENTIAL EXTRACTION AND PRECIPITATION OF THE SOLUBLE PROTEINS OF MUSCLE, WITH DATA ON THE CONCENTRATION OF THE PROTEINS IN THE MUSCLE OF THE CALF, COW, AND RABBIT. *Jour. Biol. Chem.* 61. 493–522, illus. 1924.

⁵ RITCHIE, W. S., and HOGAN, A. G. THE SEPARATION OF THE SOLUBLE PROTEINS OF RABBIT MUSCLE. *Jour. Amer. Chem. Soc.* 51. 880–886. 1929.

⁶ Unpublished data.

⁷ Howe, P. E. (See footnote 3)

⁸ Unpublished data.

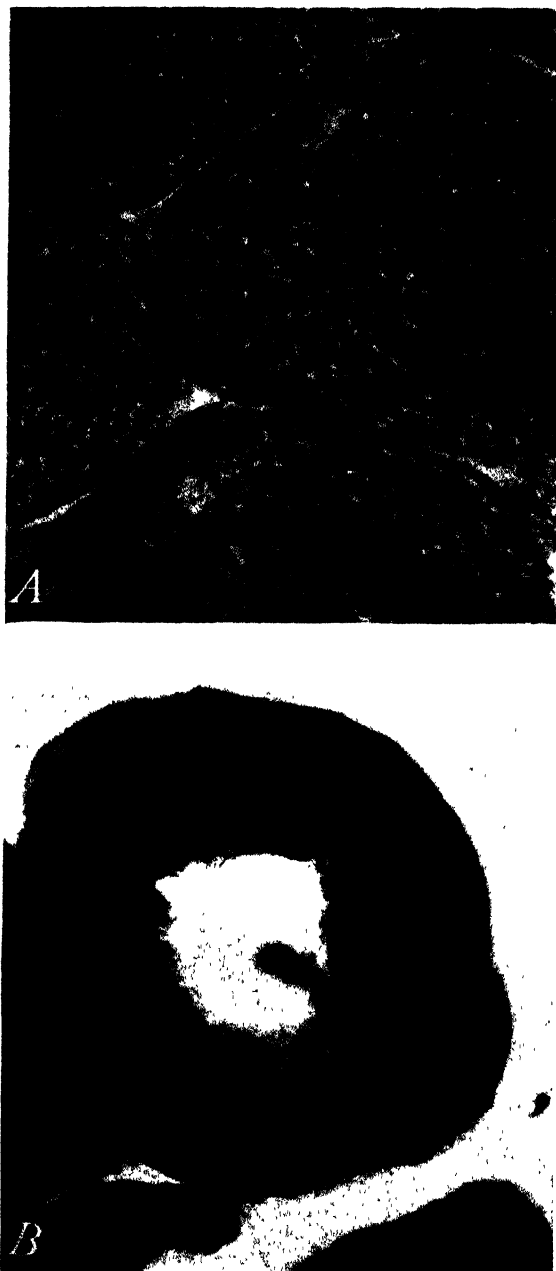


FIGURE 2.—*A*, Cross section of muscle fiber of beef showing slight pits in surface due to dissolving action of water. This is the control for comparison with the sections extracted with sodium chloride. $\times 1,250$. *B*, Muscle fiber extracted with sodium chloride 1.25 molar for 90 minutes. Sections cut at 25μ , stained with methylene blue, and mounted in balsam. Note the sarcocenter or reticular hull. Since this view is not directly down into the fiber, some of the striations may be seen on the side at right angles to the longitudinal plane. $\times 1,250$.

halides than with the phosphates, and that the maximum extraction, roughly from 60 to 80 percent of the total nitrogen, occurs at approximately a 1.25 molar concentration. Most of the work was carried out, therefore, with solutions of 1.25 molar sodium chloride and 0.225 or 0.525 molar mixture of the potassium phosphates.

Extractions were conducted at 15°C . for $1\frac{1}{2}$ hours. The temperature of extraction is not important provided the tissue proteins do not become denatured or insoluble at the temperature used. In the examination of tissue, sections were usually mounted as follows: One section after washing, mounted in glycerin on a glass slide; one section after washing with the same concentration of salt solution, stained in methylene blue and in Scharlach R; one section after washing, fixed with mercuric chloride and stained with Mann's methyl blue and eosin.

RESULTS AND DISCUSSION

The accompanying photomicrographs (figs. 2, 3, and 4) of extracted and unextracted muscle indicate the extent to which muscle structure is brought out or

modified by extraction. The two kinds of solutions, one containing a halogen salt as represented by sodium chloride and the other consisting of a mixture of phosphates, leave very characteristic pictures, corresponding in part to the degree to which protein has been extracted and in part to the specific effects they have in causing certain structural elements to swell.

The effect of extracting with sodium chloride (1.25 molar solution) is indicated in figure 2, *B*. Figure 2, *A*, a cross section of muscle tissue which has been treated in water, shows that water removes but



FIGURE 3.—Structural details of muscle fiber after extraction with 0.226 molar monopotassium phosphate and dipotassium phosphate solution. A cross section of beef muscle cut on the freezing microtome at 25μ and then extracted in the salt solution for 90 minutes. Fixed in mercuric chloride and stained with Mann's methyl blue and eosin. Mounted in balsam. $\times 400$.

a small proportion of the soluble protein of muscle. Water also has a precipitating effect upon certain proteins. There is a slight erosion of the muscle. The general effect, however, is not greatly to modify the appearance of the sections.

Sodium chloride solutions in the concentrations used have a very pronounced effect on the appearance of muscle fibers. The fibrils in many cases appear to be completely removed, leaving a much thickened rind. In thin sections the fibers appear as short tubes. Since

fibrils are no longer evident, it appears that the membranes surrounding them consist either of sarcolemma and an inner layer of tissue that readily swells in salt solutions or that the sarcolemma itself becomes



FIGURE 4.—A, Longitudinal section of muscle from leg of wasp, after extraction in a 0.525 molar potassium phosphate mixture. Fixed with mercuric chloride, lightly stained with methylene blue, and mounted in balsam. Note depleted nature of muscle fibers. $\times 2,000$. B, Longitudinal section of muscle from leg of wasp. No extraction. Used as control. Fixed with mercuric chloride lightly stained with methylene blue, and mounted in balsam. $\times 2,000$.

enormously swollen. This swelling of the muscle tissue, and especially the fiber membrane, in a strong salt solution is characteristic of the halogen salts at the concentrations the writers have used. As indi-

cated before, water has a precipitating effect upon certain muscle constituents. Higher concentrations of salts also cause precipitation. The swollen fiber membrane retains certain structural characteristics. Evidences of cross striation remain, and the outer hull seems to possess uprights or ribs lying close together.

Extraction with a mixture of potassium phosphates having a volume molar concentration of from 0.225 to 0.525 at apparently pH 7.0-7.4, is not so drastic as with the halogen salts (fig. 3). The quantity of protein removed from muscle is, as already indicated, less than that removed by the halogens. Cross sections of fiber have a very characteristic appearance. The fiber usually appears pitted, possibly because of a distortion of the bundles of fibrils in the process of extraction. Most of the supporting structure seems to be present. The ends of the fibrils are present either in the cross section or as frayed ends in the longitudinal section. Examination of a longitudinal section of the muscle of the leg of a wasp (fig. 4) shows that the thin walls of the fibrils are intact and that the material forming the cross striation remains. In some cases the walls of the fibrils appear to be partially collapsed. It would appear, therefore, that fibrils are definite structures from which a considerable quantity of material may be removed without destroying the walls or certain portions of the cross striations. The effect of extraction in bringing out the supporting structures of muscle is clearly indicated by figure 4, *A* and *B*.

Some fibers, the red ones presumably, are not so clear and transparent as the white. In these darker fibers during extraction there seems to be a diffusion of the dark coloring material, probably myohaemoglobin. Fats and lipide material seem to have been trapped in the cells and against the membranes, as shown by the staining with Scharlach R. Staining of the freezing microtome sections brings this out. Not all of it is caught in this way but certainly a considerable part is so retained.

The general effect of extraction may be a clarification of the picture of muscle structure through the removal of soluble proteins, apparently without any considerable damage to the remaining structures, or by the partial removal of some of the fibrillar structures. In either case the connective tissue elements are only slightly affected or are somewhat swollen.

SUMMARY

Studies on muscle from which as much as 60 to 80 percent of the nitrogen had been removed by extraction with neutral salt solutions, showed that the removal of the soluble proteins did not result in any material damage to the remaining structures.

In many cases the fibrils completely disappeared during the sodium chloride extraction, leaving a swollen rind of sarcolemma.

Sections of muscle extracted by sodium chloride and by phosphate mixtures were characteristically different in appearance, and the differences agreed in a general way with the quantity of proteins removed.

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